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NUTRITION REVIEWS

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NUTRITION REVIEWS

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NUTRITION AND HEMATOLOGY

Nutrition and hematology have been closely allied sciences for nearly three decades. Prior to 1920, technics of the two disciplines had been used occasionally in joint attacks on a problem. Iron metabolism had been extensively studied, and a few investigators had explored the possible effects of diet on blood formation. But interest in the nutritional aspects of blood formation and the bone marrow became much more intense after Whipple and his associates demonstrated that iron, liver, and other dietary components exert a profound influence on hemoglobin formation. A few years later, Minot and Murphy discovered the therapeutic effectiveness of liver for patients with pernicious anemia. Since then, there has been a constant stream of contributions analyzing both the nutritional requirements for normal blood formation and the nutritional disturbances underlying some of the blood dyscrasias. It is easy to understand why interest in this kind of study continues; the approach is still producing many of the most significant advances in hematology.

This type of investigation has been particularly successful in the case of macrocytic anemias. Shortly after the discovery that pernicious anemia could be controlled by liver, W. B. Castle (*Ann. Int. Med.* **7**, *2* (*1933*)) conducted his classic experiments on the pathogenesis of the disease. He found that normal human gastric juice contains an enzyme (intrinsic factor) which reacts with a dietary factor (extrinsic factor) to form a third substance, the erythrocyte maturation factor (EMF). All three of these substances are still unidentified chemically. Extrinsic factor is found in association with the vitamin B_2-complex, but is not any of the known "B-vitamins." EMF is either the active antipernicious anemia principle of liver (APA factor), or is converted to that substance by the body. One of the most exciting things about Castle's work was the way it related many morphologically similar macrocytic anemias. It was now possible to show that some were caused by a dietary deficiency of extrinsic factor, others by poor absorption from the intestinal tract, pernicious anemia itself by an inadequate secretion of intrinsic factor, and still others by a combination of the several mechanisms. All of this seemed clearcut and attention was directed primarily to the problem of identifying these various substances when pteroylglutamic acid (PGA) was synthesized and found to produce as dramatic hematologic responses in these anemias as does liver. All attempts so far to relate PGA to previously held concepts of the pathogenesis of the macrocytic anemias have failed. It is known that PGA is neither extrinsic factor nor the APA factor. No one has been able to show that it serves as a precursor of either. It is probably the prosthetic group of an enzyme possibly active in purine metabolism. It may exert its influence on the bone marrow directly and entirely independently of the APA factor, or both substances may have enzymatic activity at different levels of a chain reaction. Synthesis of PGA has stimulated several groups of investigators to redouble their efforts to isolate and identify the extrinsic and APA factors.* Even though the hema-

* This editorial was written prior to the following announcements: (1) that vitamin B_{12} had been isolated by E. L. Rickes and associates (*Science* **107**, *393* (*1948*)); (2) that vitamin B_{12} exerts a striking antipernicious anemia effect when administered in minute amounts (R. West, *Ibid.* **107**, *393* (*1948*)); and (3) that two red pigments, both of which possess antipernicious anemia activity, had been purified from liver (E. L. Smith, *Nature* **161**, *638* (*1948*)).

tologic effectiveness of PGA is currently confusing, it is not unreasonable to expect that with elucidation of its physiologic function there will develop a much more complete understanding of the macrocytic anemias and of bone marrow physiology.

Also unexplained are the following observations. In certain parts of the tropics, a type of macrocytic anemia is recognized which is thought to be nutritional in origin. The anemia apparently responds to administration of autolyzed yeast or crude liver extracts, but not to the highly purified liver preparations ordinarily used for the parenteral treatment of pernicious anemia (L. Wills, *Blood* **3,** *36 (1948)*). Furthermore, some patients with macrocytic anemia of pregnancy recover when given liver extract orally but fail to improve when extracts known to contain the APA factor are injected (*Nutrition Reviews* **3,** *327 (1945)*). It is not known whether the deficient substance is the same for both types of anemia nor whether it is PGA in either instance.

Of the PGA antagonists which have been developed during the past two years, 4 amino pteroylglutamic acid seems of particular interest. This substance produces a remarkable aplasia of the marrow (V. Minnich and C. V. Moore, *Federation Proceedings* **7,** *276 (1948)*). The effect of small doses can be prevented with PGA, but neither PGA nor liver extract can keep larger doses from producing the marrow changes. Different species of animals vary markedly in their susceptibility to 4 amino PGA. In dogs and guinea pigs the marrow changes may occur within four or five days. It is amazing that so slight a change as the substitution of an amino group in the pterin ring of PGA can change it from a vitamin to an extremely potent toxin. The observation makes one wonder whether, as a result of abnormal handling of PGA by the body, this or a similar derivative might occasionally be formed and be responsible for the production of some cases of idiopathic hypoplastic anemia.

That significant contributions have not been limited to the macrocytic anemias is evident when the following observations are recalled: (1) copper must be available in the body before iron can be used for hemoglobin synthesis; (2) cobalt deficient animals develop an anemia while those given an excess of the metal become polycythemic; and (3) deficiency of pyridoxine causes a normocytic, slightly hypochromic anemia. Other deficiencies, like those of niacin, riboflavin, and ascorbic acid have been reported to produce anemias in at least some species of animals, but the evidence in many instances is neither as complete nor as convincing as for pyridoxine. Interest recently has been attracted to a study of the nutritional factors important in protoporphyrin formation. Heavy nitrogen in the amino group and C^{14} in the alpha-carbon position of glycine have been shown to be incorporated into the protoporphyrin molecule (D. Shemin and D. Rittenberg, *J. Biol. Chem.* **166,** *621 (1946)*; K. I. Altman *et al.*, *Federation Proceedings* **7,** *2 (1948)*). J. R. Totter and associates (R. C. Steinkamp, C. F. Shukers, Totter, and P. L. Day, *Ibid.* **6,** *295 (1947)*; Totter and E. S. Sims, *Ibid.* **6,** *298 (1947)*) have suggested that PGA is involved in the production of porphyrins.

Iron metabolism is an example of a subject in which problems have multiplied despite intensive study. It is now established that human subjects absorb ferrous more efficiently than ferric iron, that iron is transported in plasma, and that only small amounts are excreted except by hemorrhage. The suggestion has been made that the intestinal mucosa absorbs or rejects the metal according to body needs and thus serves as a major regulator of iron metabolism. This attractive theory, however, by no means explains all of the known facts about iron absorption. For instance, patients with untreated pernicious anemia absorb the metal well despite adequate stores, and diets composed largely of corn grits lead to excessive

iron absorption and deposition (D. M. Hegsted, C. A. Finch, and T. D. Kinney, *Federation Proceedings* **7**, *290* (*1948*)). Many of the factors which control the utilization of iron are equally mysterious.

Studies aimed at determining the nutritional factors of importance to white blood cells have been less fruitful. It has been shown that the concentration of thiamine, niacin, PGA, and ascorbic acid is relatively high in leucocytes and tends to increase in leukemias. There is evidence that the metabolism of thiamine by leukemic cells is abnormal (*Nutrition Reviews* **1**, *84* (*1943*)). Rats with an induced PGA deficiency develop a blood dyscrasia which is characterized primarily by agranulocytosis; in some instances the leukopenia is associated with an anemia and thrombocytopenia as well. The dyscrasia can be treated successfully with crystalline PGA (*Ibid.* **4**, *91* (*1946*)). Several laboratories are currently exploring the possibility of treating leukemia, particularly myelocytic leukemia, by producing a deficiency of PGA in patients. This has been accomplished in some instances with PGA antagonists alone; in others, with antagonists plus sulfonamides given to reduce the intestinal synthesis of PGA. The results have not been too encouraging, but experience is so limited that evaluation is not yet possible. The approach is an interesting one because it represents the first physiologic attack on the treatment of leukemia.

There has been little evidence to suggest that trace elements and other metals are of specific importance for leucocyte metabolism, but the subject is just beginning to be explored. Zinc, for instance, is present in human white blood cells in a concentration twenty-five times greater than in red cells; in dogs, the differential is 100:1 (B. L. Vallee, R. G. Fluharty, and J. G. Gibson, *Proc. Fourth International Cancer Res. Congress, p. 137* (*1947*)). Furthermore, the zinc content of leukemic white blood cells is markedly below normal, but tends to rise when the leukemia is treated (J. G. Gibson, B. L. Vallee, R. G. Fluharty, and J. E. Nelson, *Ibid., p. 104* (*1947*)). Studies with radioactive zinc indicate that the metal is conserved by being re-utilized. A rhythmic rise and fall in the Zn^{65} content of the cells with cycles of from five to eight days have been interpreted as indicating that these cycles indicate the length of life of leucocytes. Use of radioactive isotopes may well eliminate many of the technical difficulties which have prevented this type of study in the past.

Discovery of the importance of vitamin K for the formation of prothrombin has unquestionably been the most important contribution of nutrition to our understanding of the coagulation mechanism. Still to be determined, however, is whether vitamin K catalyzes prothrombin synthesis or serves as a precursor of the molecule. Of equal importance to coagulation in the prevention of abnormal blood loss is the integrity of the capillary wall. The role of ascorbic acid in helping to preserve normal capillary resistance has been established. Claims have also been made for the existence of vitamin P and for its importance in capillary function, but the evidence has not been entirely convincing.

An attempt has been made to emphasize that nutritional studies have contributed significantly to all phases of hematology. In this short space it has been utterly impossible even to mention all of the important advances; the investigations cited are intended to serve only as examples. While emphasis has been given to the contributions made by using nutritional approaches to hematologic problems, the thesis might as easily have been reversed. Hematologic technics have been equally important in the study of deficiency states. The sciences of hematology and nutrition have been truly interdependent; and there is every reason to think that their close association will continue to be scientifically profitable for years to come.

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FACTORS AFFECTING RESISTANCE OF TEETH TO DECAY

Studies describing reductions in the susceptibility of Norwegian and English children to tooth decay during the war have been reviewed in this journal (*Nutrition Reviews* **4,** *142 (1946)*). In the former case, the investigator who made the survey suggested that the dramatic reduction in dental caries experience could be attributed to the drastic restriction in sugar and other refined carbohydrate foods. On the other hand, the decrease in caries in London schoolchildren was attributed by those responsible for the survey, to public health measures to improve the general nutritional status of the children. The relation of diet to tooth decay constitutes a complex problem because of the difficulties in evaluating the respective roles of the oral environment and of systemic influences both during and after tooth development. The above data served mainly to indicate the need for further study of the wartime caries reduction and to stimulate the collection and exhaustive evaluation of additional related data.

R. F. Sognnaes (*Science* **106,** *447 (1947)*) has presented a clinical and experimental study which tends to throw doubt upon the sufficiency of oral environmental factors in the etiology of dental caries. A clinical analysis of certain well established trends in dental caries incidence which have occurred in European children during the past fifty years was based on the dental caries experience of about 800,000 children who had been surveyed by various investigators in eleven European countries. An attempt was made to correlate marked wartime caries reductions with the drastic reductions in the amounts of refined carbohydrates, and in particular, with the amount of sucrose available for human consumption during both World Wars. There was a time relation between the decrease in refined carbohydrates and the reduction in dental caries incidence which could not be satisfactorily explained on the basis of any rapid or concurrent mechanism operating through the oral environment. The initial reductions in caries incidence did not occur immediately and maximum reduction was not observed until a period of several years had elapsed. This point was best demonstrated by a chart which presented the reduction in sugar consumption and the percentage reductions in the number of carious permanent teeth in groups of 7 year old Norwegian children during each year of the war. Seven year old children were selected so that all permanent teeth being considered would be newly erupted ones with a comparably short period of oral exposure. Children of this age group might have as many as four permanent incisors besides their four first permanent molars, all of which had erupted during the year previous. In this way, the length of oral exposure and the caries incidence in permanent teeth could be fairly compared in the 7 year old group of children for each succeeding year. As read from the chart, the reduction in caries incidence from 1939 to 1940 and 1941 was insignificant, i. e., of the order of 5 per cent only; and by 1942, about 23 per cent only. In 1943, the caries reduction had reached 43 per cent, and in 1944, 57 per cent, but the most significant reduction recorded was not until 1945, when a 75 per cent decrease was noted, i.e., in the 7 year olds born at the beginning of the war. The percentage reduction in sugar consumption did not concur with this caries curve. Sugar rationing was begun in 1939 at 50 per cent reduction and did not go beyond 65 per cent reduction following the invasion by the German armies in 1940. This relative constancy of the sugar supplies available for human consumption is in distinct contrast to the caries curve for the period from 1941 to 1945, which showed a sharply decreasing incidence of caries from year to year. From these data, it is obvious that, after several years of wartime dietary regimens, dramatic increases in the re-

sistance of the newly erupted teeth to tooth decay had occurred and that there had not been simultaneous parallel decreases in sugar consumption as the war progressed. Since these teeth had erupted during the year previous for each group of 7 year olds, the exposure to the oral environment had been of the same duration for each group, and represented only a brief portion of the total wartime period. If sugar consumption, as reflected in the oral environment, were the primary factor influencing the production of tooth decay, and if tooth structure and metabolism were of no consequence, then the comparable exposure of the teeth of the various 7 year old groups to comparable oral environments would not be expected to result in such a markedly greater caries reduction from year to year from 1939 to 1945. The much greater reductions in 1943 to 1945 strongly suggests that there had been "an accumulative favorable effect before the teeth erupted into the oral environment." This mechanism could not be attributed to any obvious, over-all uniform increase in the consumption of any previously demonstrated caries-inhibiting food or food factor. It was suggested that the mechanism might be related to the high consumption of natural foods, potatoes, cabbage, and wartime bread which contained 80 to 95 per cent of the whole grain.

In a study with mice, hamsters, and the Long-Evans strain of rats, Sognnaes (*loc. cit.*) has been able to produce carious lesions similar to those in man by feeding the highly purified diets, complete in all known nutritional essentials which have been demonstrated to produce caries in the cotton rat (*Nutrition Reviews* **3,** *83* (*1945*)). In the latter species, carious lesions developed readily in the molars when the ration was fed from weaning at 14 days for an experimental period of fourteen weeks. By way of contrast, carious lesions did not develop in mice and Long-Evans rats, and was limited in hamsters when the maintenance of these animals on purified rations did not begin until weaning at 21 days, at which time tooth development in these species is largely completed. When purified rations were fed to the dams of these species from the time the litter was delivered until weaning and to their offspring after weaning, the incidence of carious lesions in the offspring was much higher than in offspring fed the purified rations after weaning but whose dams had been fed rations composed of natural foodstuffs during pregnancy and lactation. Further, if the purified rations were fed to the females prior to the beginning of gestation, their offspring, when fed the purified rations after weaning, had an even higher susceptibility to the development of carious lesions indicating that a further depletion of the materials necessary for the development of caries resistant teeth had occurred. The illustration of the extent to which these experimental manipulations altered caries susceptibility concerned the hamster. The ratio of caries incidence as expressed in terms of carious molars of three groups of hamsters whose exposure to the purified ration was commenced before, during, and after tooth development respectively, was 20:10:2.

The data presented and evaluated by Sognnaes are based on sufficiently large groups of children to demonstrate adequately the significant trends in caries susceptibility which have occurred in European children during the last twenty years. The demonstration that there is an interval of several years between drastic reductions in availability of sucrose and other refined carbohydrate foods and the attainment of the maximum reduction in dental caries incidence merits the postulate that there is a favorable effect of diet during tooth development on the resistance of teeth to tooth decay. Of equal importance is the evidence in laboratory animals that the resistance to tooth decay can be influenced by the diet consumed during tooth development. The latter finding provides an assay whereby careful experimentation

can be conducted to determine whatever factors in the diet of experimental animals affect the development of resistance in teeth. Through this mechanism, it may become possible to interpret the effects of wartime dietary regimens more exactly than is now conceivable.

Of interest to American investigators is the fact that apparently no comparable data are available for the dental caries incidence of the children of Canada and United States. The early development of school dental clinics in European countries and the systematic collection of yearly records of dental caries incidence have resulted in large masses of data, the value of which is now becoming evident to dental investigators. It would be of inestimable value if comparable records could be obtained for the past fifty years for American children. While the sucrose consumption was restricted by rationing during both wars, there was not any major restriction in the consumption of other refined carbohydrate foods except for limited periods of time or intermittently on a local rather than on a national basis.

One of the most interesting continuations of this problem will be the yearly data on dental caries incidence of European children to determine whether the maximum dental caries reductions had been attained by 1945, to determine how long these drastic reductions in caries incidence continue and, if possible, to correlate any future changes in caries incidence with dietary alterations.

BIOLOGIC SYNTHESIS OF PROTEINS

It has not been difficult to demonstrate the *in vitro* degradation of the major foodstuff materials in tissue preparations since thermodynamically this is at least in the direction of a decrease in free energy. However, synthesis of carbohydrate, fat, and protein requires energy and it has not been as easy to elucidate the mechanism of synthesis of these three major biochemical components. For glycogen synthesis, it has taken many years of intensive effort, with purification of the enzymes involved, to recognize the steps involved. Progress in the understanding of fat synthesis is only beginning. In the case of protein synthesis, perhaps the most baffling because of the great complexity and number of proteins that exist in a single organism, we are largely in the speculative stage. Yet there are many contributions with an interesting bearing on this problem.

Earlier studies on the synthesis of protein-like substances ("plastein") from concentrated proteolytic digests in emulsions and in the presence of pepsin will be recalled. Such a result, although the product of synthesis was not a typical protein, was interpreted as the approach to equilibrium from the direction of the products of proteolysis. For some time it had to be assumed that the intracellular proteases in conjunction with intact cellular structure might favor synthesis of protein from the amino acids. A possible analogy to this condition is found in the work of Bergmann and associates who have demonstrated the reversibility of the chymotrypsin reaction by synthesizing a tyrosine-glycine peptide from appropriate derivatives of the amino acids. In this case the product of the synthesis was insoluble and thus removed from the chemical equilibrium, which permitted the synthetic reaction to proceed. Cellular structure, by adsorption mechanisms, might accomplish a similar end result by removal of the synthetic product.

It has been generally recognized that the process of protein synthesis requires energy, but the means of supplying "biological energy" outside the cell had not yet been recognized. The concept of "energy-rich phosphate bonds" (F. Lipmann, *Advances in*

Enzymology **1,** *99 (1941)*) and of adenosinetriphosphate as a fundamental source of biologic energy in many biologic processes, gave new impetus to the study of biologic synthesis in general. At about the same time the concepts of R. Schoenheimer ("The Dynamic State of Body Constituents," *Harvard University Press (1942)*) were developed. Employing radioactive tracers it appeared that proteins were not static compounds, but were dynamic in that amino acids in the protein molecule could be readily exchanged with free amino acids. Either amino acids could be incorporated into preformed proteins, or if complete degradation and synthesis were necessary for such incorporation, then this process is very rapid.

As in other biochemical investigations, advantage has been taken of model systems. Thus, P. P. Cohen and R. W. McGilvery (*J. Biol. Chem.* **166,** *261 (1946)*; **169,** *119 (1947)*; **171,** *121 (1947)*) have utilized the formation of para-aminohippuric acid from para-aminobenzoic acid and glycine by liver slices and homogenates as a model for peptide bond synthesis. It is recognized that the —C(=O)—NH— linkage formed is not entirely typical of the peptide linkage in proteins, although it had been suggested that the formation of hippuric acid resembles peptide synthesis both chemically and thermodynamically. Nevertheless this is a possible restriction of the model system employed. In their first paper the investigators found that the peptide-linked para-aminohippuric acid is formed from para-aminobenzoic acid and glycine by liver and kidney slices. Of special interest was the fact that the synthesis did not proceed anaerobically or in the presence of well known oxidation inhibitors such as cyanide, arsenite, fluoride, and iodoacetate. This implied that the synthesis was not simply through reversal of an enzymatic hydrolytic process, but that the energy required for the synthesis was supplied by oxidative processes, possibly through a high-energy phosphate intermediate. In their second paper, crude homogenates were utilized and the expected complications of inorganic ions, pH, and tissue dilution had to be considered. Nevertheless the synthesis was demonstrated, and the need for additional cytochrome-c, an oxidation catalyst, was demonstrated. Adenosinetriphosphate was found to support the reaction anaerobically, but to a small degree as compared with the stimulation of synthesis afforded aerobically by citrate, succinate, fumarate, malate, and oxalacetate. The oxidation of the latter compounds is known however to produce energy-rich phosphate, and in experiments with washed liver homogenates, the indispensability of adenosinetriphosphate for the peptide bond synthesis was made clear. The work of Cohen and McGilvery is important in demonstrating the need for oxidative energy in the synthesis of at least this model peptide bond. They suggest that further steps should be directed toward the study of phosphate transfer from adenosinetriphosphate to direct intermediates in the synthesis. Presumably these intermediates are the amino acids, and the process of protein synthesis would become analogous to the formation of glycogen from glucose-1-phosphate, involving successive phosphoric acid removals.

Closely related to the above studies is that of N. O. Kaplan and F. Lipmann (*Federation Proceedings* **6,** *266 (1947)*) in which the synthesis of acetyl-sulfanilamide from sulfanilamide and acetic acid in a purified liver enzyme system was found to require adenosinetriphosphate. Here also the peptide linkage formed is not identical with that in proteins, but may be analogous.

Pertinent to the general problem is the work of F. Friedberg, T. Winnick, and D. M. Greenberg (*J. Biol. Chem.* **169,** *763 (1947)*) who demonstrated the incorporation of C^{14} labeled glycine into the dipeptide leucylglycine in the rat. Radioactive glycine and ordinary L-leucine were adminis-

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As in other biochemical investigations, advantage has been taken of model systems. Thus, P. P. Cohen and R. W. McGilvery (*J. Biol. Chem.* **166,** *261 (1946)*; **169,** *119 (1947)*; **171,** *121 (1947)*) have utilized the formation of para-aminohippuric acid from para-aminobenzoic acid and glycine by liver slices and homogenates as a model for peptide bond synthesis. It is recognized that the —C(=O)—NH— linkage formed is not entirely typical of the peptide linkage in proteins, although it had been suggested that the formation of hippuric acid resembles peptide synthesis both chemically and thermodynamically. Nevertheless this is a possible restriction of the model system employed. In their first paper the investigators found that the peptide-linked para-aminohippuric acid is formed from para-aminobenzoic acid and glycine by liver and kidney slices. Of special interest was the fact that the synthesis did not proceed anaerobically or in the presence of well known oxidation inhibitors such as cyanide, arsenite, fluoride, and iodoacetate. This implied that the synthesis was not simply through reversal of an enzymatic hydrolytic process, but that the energy required for the synthesis was supplied by oxidative processes, possibly through a high-energy phosphate intermediate. In their second paper, crude homogenates were utilized and the expected complications of inorganic ions, pH, and tissue dilution had to be considered. Nevertheless the synthesis was demonstrated, and the need for additional cytochrome-c, an oxidation catalyst, was demonstrated. Adenosinetriphosphate was found to support the reaction anaerobically, but to a small degree as compared with the stimulation of synthesis afforded aerobically by citrate, succinate, fumarate, malate, and oxalacetate. The oxidation of the latter compounds is known however to produce energy-rich phosphate, and in experiments with washed liver homogenates, the indispensability of adenosinetriphosphate for the peptide bond synthesis was made clear. The work of Cohen and McGilvery is important in demonstrating the need for oxidative energy in the synthesis of at least this model peptide bond. They suggest that further steps should be directed toward the study of phosphate transfer from adenosinetriphosphate to direct intermediates in the synthesis. Presumably these intermediates are the amino acids, and the process of protein synthesis would become analogous to the formation of glycogen from glucose-1-phosphate, involving successive phosphoric acid removals.

Closely related to the above studies is that of N. O. Kaplan and F. Lipmann (*Federation Proceedings* **6,** *266 (1947)*) in which the synthesis of acetyl-sulfanilamide from sulfanilamide and acetic acid in a purified liver enzyme system was found to require adenosinetriphosphate. Here also the peptide linkage formed is not identical with that in proteins, but may be analogous.

Pertinent to the general problem is the work of F. Friedberg, T. Winnick, and D. M. Greenberg (*J. Biol. Chem.* **169,** *763 (1947)*) who demonstrated the incorporation of C^{14} labeled glycine into the dipeptide leucylglycine in the rat. Radioactive glycine and ordinary L-leucine were adminis-

tered, and the peptide isolated with the aid of an injection of leucylglycine prior to killing the animal. K. Bloch and H. S. Anker (*Ibid.* **169,** *765 (1947)*) have taken advantage of the naturally occurring and readily isolated peptide, glutathione, to demonstrate peptide synthesis in rat liver slices. Glycine labeled with N^{15} was incubated with rat liver slices, and glutathione was added to aid in the isolation of glutathione. The isolation and analysis of the glutathione demonstrated the incorporation of the radioactive glycine into the glutathione. Finally, employing homogenized liver and spleen, F. Friedberg, T. Winnick, and D. M. Greenberg (*Ibid.* **171,** *441 (1947)*) have claimed to demonstrate the incorporation of labeled glycine into protein. The isolation and preparation of the protein was not described in this paper, and the incorporation of radioactive glycine occurred to the extent of only 0.1 per cent of that present in the medium. It is of interest however that the medium used to demonstrate this incorporation was that of Cohen and McGilvery, implicating the necessity of oxidative metabolism and energy-rich phosphate compounds. Somewhat more thoroughly described are the experiments of I. D. Frantz, R. B. Loftfield, and W. W. Miller (*Science* **106,** *544 (1947)*) in which C^{14} OOH labeled alanine was found to be incorporated into the proteins of liver slices on incubation. The trichloracetic acid precipitated proteins were washed and hydrolyzed, and the carbon dioxide liberated by the ninhydrin reaction was measured for C^{14}. Little or no incorporation of alanine occurred in the absence of oxygen, again implicating the need for oxidative energy.

Of special importance is the mechanism by which the energy of oxidation or metabolism is utilized in the synthesis of protein, and of ultimate importance is the nature of the directing force which permits the synthesis of so many different specific protein molecules. In the case of polysaccharide synthesis the energy from metabolism is incorporated in the glucose-1-phosphate molecule. In the case of protein synthesis, S. Spiegelman and M. D. Kamen (*Science* **104,** *581 (1946)*) have reviewed their work to show that during the growth of yeast there is a flow of phosphorus from the nucleoprotein phosphorus fraction. This was accomplished by growing the yeast in a medium containing P^{32} phosphate for forty-eight hours, which permitted equilibration of all the phosphorus fractions with P^{32}. When such cells were washed and allowed to ferment glucose, under which conditions there was no growth or budding, the P^{32} activity of the nucleoprotein fraction remained constant. The results differed when growth was permitted by adding ammonia to the medium or by forcing enzyme synthesis by addition of maltose to the medium. Under these conditions there was a decrease to nearly one third of the P^{32} activity of the nucleoprotein fraction. It was therefore suggested that the nucleoproteins are the specific energy donators for the reactions involved in protein and enzyme synthesis. The same investigators have suggested that the "self-duplicating" units controlling the specificity of the type of protein synthesized are also nucleoproteins. Presumably these units arise eventually from the genes in the cell nucleus which control the synthesis of the various specific proteins. These self-duplicating units are the patterns or templets for duplication of successively synthesized protein molecules. Other accepted self-duplicating units, for example viruses and genes, are also nucleoproteins.

D. Wrinch (*Science* **106,** *73 (1947)*) has also discussed the biologic synthesis of proteins. She stresses the importance of the surface structure of the protein molecule and compares the structure to that in a mineral crystal. A theory of biosynthesis was proposed in which replication of protein molecules is similar to crystal growth. It is further suggested that the whole molecule need not be replicated, but that proteins are composed of smaller units, still characteristic of the whole protein. These units have been

termed "proteons," and these may be the active patches which serve as the mold or templet for synthesis of new units. It is reminded that the smaller proteon units are not simply theoretical; the lactalbumin precursor, certain digestion products of beef pseudoglobulin, beta-lactoglobulin, casein, and silk appear to be low molecular units with some characteristics of the original protein. The dissociation of hemoglobin into smaller units in urea solutions will be recalled. Wrinch's theory of protein biosynthesis thus postulates the existence of simpler protein intermediates, whose composition and structure may provide a simpler objective in the problem of protein synthesis.

It is evident that the attack on the general problem of protein synthesis has begun from several angles, and it cannot be denied that a variety of approaches will be needed for solution of this most fundamental of problems.

PANTOTHENIC ACID AND ADRENAL CORTICAL ACTIVITY

Evidence of a relation between pantothenic acid deficiency and the adrenal cortex has been accumulating since F. S. Daft and W. H. Sebrell (*Pub. Health Reports* **54,** *2247 (1939)*) called attention to adrenal cortical hemorrhage and necrosis in pantothenic acid deficient animals. It has since been established that deficiency of this factor is associated with a zonal type of lipoid depletion of the adrenal cortex. The lipoid content of the zona glomerulosa remains normal while the zona fasciculata loses its lipoid material rapidly and eventually completely (H. W. Deane and J. M. McKibbin, *Endocrinology* **38,** *385 (1946)*; A. C. McQueeney, L. L. Ashburn, F. S. Daft, and R. Faulkner, *Ibid.* **41,** *441 (1947)*). After six weeks of pantothenic acid deficiency the adrenal cortices of young rats give completely negative tests for the presence of ketosteroids. No significant increase or decrease in the weights of the adrenals is found when compared with those of paired-fed or controls fed ad libitum. Other confirmed observations, from the anatomic point of view, include great diminution in the weights of the thymus and lymph nodes due, in part, to disappearance of the lymphoid elements from these structures. Five days of treatment with pantothenic acid produces a prompt and striking return of adrenal cortical lipids and a return toward normal of thymus and lymph glands.

Attempts to correlate pantothenic acid deficiency with adrenal cortical function have led one group (Deane and McKibbin, *loc. cit.*) to conclude that the anatomic changes observed are explainable on the basis of increased functional activity of the cortex. They point out that cortical lipoid depletion and involution of lymphoid tissues are nonspecific alterations which may be produced by a wide variety of "alarming stimuli" (Selye). They regard pantothenic acid deficiency as comprising a stimulus to increased production of adrenocorticotropic hormone. This results in increased production and release of cortical lipids (the latter being responsible for lymphatic involution) with eventual exhaustion of the lipid content of the gland. Although increased production of adrenocorticotropic hormone or adrenocortical steroids has not been demonstrated in pantothenic acid deficiency, the reasoning is consonant with the concept of H. Selye (*J. Clin. Endocrinology* **6,** *117 (1946)*).

On the other hand, G. C. Supplee, R. C. Bender, and O. J. Kahlenberg (*Endocrinology* **30,** *355 (1942)*) and R. Gaunt, M. Liling, and C. W. Mushett (*Ibid.* **38,** *127 (1946)*) have interpreted their results as indicating adrenal cortical insufficiency in pantothenic acid deficiency. The former observed that pantothenic acid deficient rats consume larger quantities of salt when on a self selection salt intake than do treated controls, while

the latter group of workers found that pantothenic acid deficient animals show much less resistance to water intoxication than do normal animals. These observations together with the sudden prostration and terminal dehydration often seen in pantothenic acid deficient animals led others to study electrolyte metabolism in such preparations. M. M. Wintrobe *et al.* (*Bull. Johns Hopkins Hosp.* **73,** *313 (1943)*) observed no deficiency of sodium conservation in pantothenic acid deficient pigs. McQueeney *et al.* (*loc. cit.*) using pantothenic acid deficient rats found no defect in sodium conservation despite drastic restriction of sodium intake. In this connection it should be mentioned that while adrenalectomy produces loss of ability to conserve sodium, hypophysectomy is associated with little loss of this ability, but with great loss of those functions controlled by adrenal ketosteroids. It is conceivable that in pantothenic acid deficiency there exists a decrease of ketosteroid activity, and yet a sufficiency of desoxycorticosterone-like hormones. This is compatible with the finding of normal lipoid content of the zona glomerulosa and lipoid depletion of the zona fasciculata in pantothenic acid deficient animals.

The two points of view (cortical hyperfunction versus cortical hypofunction) do not seem to be irreconcilable. It is likely that the "alarm reaction," which is associated with the release of large amounts of adrenal cortical steroids, is the initial response of the animal to pantothenic acid deficiency. This phase of the total response is associated with involution of lymphoid structures. If the alarming stimulus is sufficiently prolonged, adrenal cortical exhaustion supervenes and a terminal state of adrenal cortical insufficiency occurs. The final association of lymphoid involution and adrenal cortical insufficiency could be related to the continued low intake of food (associated with pantothenic acid deficiency) in not providing essential elements for reconstruction of lymphoid tissue.

UTILIZATION OF PHYTIN

Phytin is the calcium-magnesium salt of phytic acid, the hexaphosphoric acid ester of inositol. It is an important compound because of its inositol moiety and because a large part of the phosphorus of cereal seeds is present in this organic form. For the latter reason there has been much interest in the dietary factors concerned with the utilization of phytic acid phosphorus. Its availability depends to a large extent on dietary supplies of calcium and of vitamin D (C. H. Krieger and H. Steenbock, *J. Nutrition* **20,** *125 (1940)*). Calcium phytate is insoluble and is poorly absorbed in the rat if an excess of calcium is present, i.e., if the calcium-phosphorus ratio is 2 or more. An enzyme, phytase, is present in cereals and in animal tissues and it is believed that it normally hydrolyzes phytin to inositol and to phosphoric acid or its salts. Phytase in soybean meal is not essential for the utilization of the phosphorus of the phytin in the meal (R. R. Spitzer and P. H. Phillips, *Ibid.* **30,** *183 (1945)*). Present evidence does not indicate whether phytic acid, or its salts, is hydrolyzed during absorption by a phytase in intestinal mucosa or prior to absorption by a phytase in an intestinal secretion. The relation of phytin to the occurrence of rickets in man has been discussed in previous reviews (*Nutrition Reviews* **3,** *61 (1945)*; **4,** *351 (1946)*).

The nonavailability of phytic acid phosphorus in diets deficient in vitamin D has not been explained and it is not known whether vitamin D is effective by facilitating the absorption of phytic acid, by stimulating the formation of phytase, or by enhancing the activity of the enzyme. The possibility that this vitamin may be essential for the forma-

tion and activity of phytase has been investigated by R. R. Spitzer, G. Maruyama, L. Michaud, and P. H. Phillips (*J. Nutrition* **35,** *185 (1948)*).

These workers compared the effects of vitamin D on bone development and on phytase activity in young rats fed diets in which most of the calcium and phosphorus was supplied either in the form of inorganic salts or as calcium phytate. The total calcium and phosphorus amounted to 0.6 and 0.25 per cent, respectively, of the diets. Determinations of bone ash in the femurs and of phytase activity in homogenates of portions of the small intestine were made after an experimental period of thirty-two days. The bone ash in rats receiving inorganic calcium and phosphorus amounted to 47.1 per cent of the bone, a value that showed good bone formation and that was improved only slightly in rats receiving a supplement of vitamin D. This was in marked contrast to the bone ash level of 23.3 per cent in rats on the vitamin D deficient calcium phytate diet. These rats were markedly rachitic. Supplementation with vitamin D prevented rickets and increased the utilization of the calcium and phosphorus of calcium phytate as evidenced by a bone ash level of 43.4 per cent. Growth was significantly impaired on both phytate diets. Although bone development was definitely subnormal in the rachitic rats fed calcium phytate as the principal source of calcium and phosphorus, no decrease in the activity of intestinal phytase was demonstrable. Furthermore, the addition of vitamin D to tissue homogenates in *in vitro* experiments did not affect the phytase activity.

Despite improved utilization of calcium and phosphorus in the vitamin D supplemented diets containing calcium phytate, the rats in this group made the poorest gains in weight and developed a severe alopecia. The pattern of the hair loss was similar to that of inositol deficiency, previously described by T. J. Cunha, S. Kirkwood, P. H. Phillips, and G. Bohstedt (*Proc. Soc. Exp. Biol. Med.* **54,** *236 (1943)*) and by R. R. Spitzer and P. H. Phillips (*Ibid.* **63,** *10 (1946)*). It is difficult to correlate the alopecia observed by Spitzer *et al.* with a lack of inositol because the diet contained 0.1 per cent of added inositol in addition to that presumably liberated by the hydrolysis of phytic acid. These authors suggest that an intermediate substance, antagonistic to inositol, may have increased the requirement of inositol. Evidence for an anti-inositol has been reported (*Nutrition Reviews* **4,** *351 (1946)*).

These data throw no light on the factors involved in the utilization of phytic acid. On the contrary, the ramifications of the whole problem will be greatly extended if the abnormality present in the animals receiving vitamin D and calcium phytate can be definitely shown to be the result of inositol deficiency. The significance of phytin in the plant world and of inositol, or of its derivatives, in the animal organism is still obscure. Phytic acid affects the absorption of iron (*Nutrition Reviews* **2,** *45 (1944)*) and influences the solubility characteristics of protein (*Ibid.* **5,** *91 (1947)*). It is entirely possible that its role in metabolism and its dietary importance have been underestimated.

INTRINSIC FACTOR IN THE HUMAN STOMACH

Organotherapy has been used in the treatment of human diseases for many centuries. Pellagra in adults has been treated satisfactorily with stomach preparations (T. D. Spies, *J. Clin. Invest.* **13,** *807 (1934)*) and when gastric juice from normal persons is administered it produces a remission of symptoms of pellagra while the individuals are still on a "pellagra-producing diet" (V. P. Sydenstricker, E. S. Armstrong, C. J.

Derrick, and P. S. Kemp, *Am J. Med. Sci.* **192,** *1 (1936)*). Interestingly enough, gastrectomy in some animals produces a pellagra-like syndrome which is not ameliorated by niacin but responds to treatment with preparations of stomach (S. Petri, F. Nørgaard, and J. Bing, *Ibid.* **195,** *717 (1938)*). A deficiency disease syndrome occurring in infants and young children has been successfully treated with a preparation of powdered stomach, whereas liver extract, vitamins, and yeast were of no value (*Nutrition Reviews* **4,** *52 (1946)*).

Just why the state of the gastric mucosa plays an essential role in prevention of pernicious anemia is still unexplained. The so-called argentaffin cells occur in the cardial and pyloric portions of the gastric mucosa and also in the intestine. Some recent work suggests that these cells contain pterin and may, in part, be responsible for the hematopoietic activity of the gastric mucosa (*Nutrition Reviews* **4,** *270 (1946)*).

Since Castle's classic work demonstrating the necessity of the intrinsic factor for normal regeneration of the blood, investigators have sought the exact source and nature of this intriguing compound. In pernicious anemia, histologic studies show that it is the fundal portion of the stomach, that is, the portion containing the fundal glands, which undergoes the most conspicuous changes (A. J. Cox, *Am. J. Path.* **19,** *491 (1943)*; H. A. Magnus and C. C. Ungley, *Lancet* **I,** *420 (1938)*). These changes are atrophic and degenerative in character. The area of the pyloric glands is either unchanged or does not present pronounced changes.

Naturally, various areas of the animal stomach have been prepared for administration to the human patient with pernicious anemia. Studies have shown that the pyloric portion of the hog stomach is effective in treatment of human pernicious anemia, while the fundal portion is not. Preparations of the cardial portion of the stomach and the duodenum of the hog also are effective (E. Meulengracht, *Acta med. Scandinav.* **82,** *352 (1934)*; **85,** *50 (1935)*; *Am. J. Med. Sci.* **197,** *201 (1939)*). Others, however, have found that the fundal portion of the hog stomach is active in the antipernicious anemia factor (S. Petri, O. Bang, W. Kiaer, and A. K. Nielsen, *Acta med. Scandinav.* **116,** *273 (1944)*).

The early work of Meulengracht was later confirmed by H. J. Fox and W. B. Castle (*Am. J. Med. Sci.* **203,** *18 (1942)*). The investigators reported that the pyloric portion of the hog stomach is active, whereas the fundal portion is not. They also studied the antipernicious anemia effect of treatment with preparations of the fundal glands of the human stomach and dried pylorus of human stomach in contrast to results obtained from animal stomachs. The preparation of human fundus showed a maximal effect on pernicious anemia in 2 patients. One patient, however, showed a slight increase of reticulocytes on administration of the preparation of human pylorus. Fox and Castle pointed out that because of the distribution o pyloric and fundal glands in the human stomach the pyloric preparations were undoubtedly not pure but contained an admixture of fundal glands.

The pyloric-fundal gland border in the human stomach has been described by E. Landboe-Christensen (*Acta path. et microbiol. Scandinav.* (*Suppl.*) **54,** *671 (1944)*). By utilizing this mapping of the glandular zone, Landboe-Christensen and C. M. Plum (*Am. J. Med. Sci.* **215,** *17 (1948)*) have been able to separate the pyloric and fundal areas anatomically and can thus, they believe, employ rather pure pyloric and fundal preparations for therapeutic testing.

A striking therapeutic effect was produced when 100 g. of dried, powdered, and defatted stomach wall from the anatomic fundal part of the human stomach were fed to patients with pernicious anemia. This is in agreement with the observation of Fox and Castle, and is in marked contrast to the total

ineffectiveness of preparations of the fundal portion of the stomach of the hog. An 80 g. preparation of dried stomach from the pyloric part of the human stomach was fed to a second patient with pernicious anemia and moderate improvement was noted. The preparation was effective despite the fact, stated by the authors, that no glands of the fundal type were present in the preparation.

This work suggests that the antipernicious anemia activity of the human stomach occurs not only in association with human fundal glands but also is probably associated with human pyloric glands.

NUTRITIONAL STATUS AND INFECTION RESPONSE

With the aim of gaining information concerning the relation of nutritional status to the response to infection, studies have been initiated in the Department of Nutrition at the Harvard School of Public Health. The first report (J. Metcoff, D. B. Darling, M. H. Scanlon, and F. J. Stare, *J. Lab. Clin. Med.* **33,** *47 (1948)*) describes the host hematopoietic, immunologic, and plasma protein responses to infection with Salmonella typhimurium, as these responses were influenced by two contrasting levels of protein intake.

The studies were carried out in rats, half of which received an adequate diet containing 18 per cent casein while the remainder were fed a diet containing only 2 per cent casein. Approximately half of the well fed and of the protein depleted rats were infected with virulent S. typhimurium. Rats on adequate nitrogen intake consumed approximately 155 to 368 mg. per 100 g. rat per twenty-four hours whereas those subjected to severe protracted protein deficiency had nitrogen intakes varying from 19 to 39 mg. per 100 g. rat per twenty-four hours. The study was carefully planned, well controlled, and carried out in 60 rats. Total blood volume was measured and electrophoretic studies of the plasma were made, in addition to blood cultures, agglutinin titers, and blood and bone marrow morphologic examinations.

In these studies, the total circulating plasma proteins, particularly the gamma globulins, were relatively uninfluenced by infection *per se* in both well nourished and protein deficient animals despite observed changes attending protein deficiency. With protracted protein deficiency, the circulating plasma proteins per unit of surface area were decreased. Infection was associated with a slight reduction in the animals fed adequate protein, as compared with the controls, but both groups of deficient animals had similar unit circulating protein levels. The decreased unit circulating plasma proteins appeared to be due to a depletion of albumin and gamma globulin components, independent of infection.

In the presence of infection there was decreased circulating hemoglobin per unit of surface area both in the well nourished and in deficient infected rats. The diminution was somewhat more marked in the protein deficient animals. The total leucocyte counts of the deficient groups were somewhat lower than those of the well nourished rats. The initial postinfection relative lymphopenia manifested by both infected groups was similar, however. Protein deficiency was associated with a tendency toward a relative increase of immature myeloid forms in the femoral bone marrow and decreased numbers of maturing erythroid elements. Well nourished infected animals appeared to have a relatively greater number of maturing erythroid forms than the controls. Dietary deficiency appeared to influence the bone marrow more than infection.

No significant difference in the incidence of bacteremia was noted between infected rats on the 2 per cent or the 18 per cent casein diets. Despite relative quantitative

depletion of circulating gamma globulin, protein deficient rats attained antibody titers equivalent to those of the well nourished animals in response to infection. With the object of observing the effects of protein deficiency on the immune and anamnestic response, twenty-eight days after infection all animals were injected subcutaneously with S. typhimurium O antigen. Following such specific secondary antigenic stimulation the rise in humoral antibody titer was more marked in the deficient than in the well nourished rats. Despite a rise in titer no significant change in circulating gamma globulin was observed in either group. Thus bacteriologic and immune responses resulting from infection were essentially unaltered by severe dietary protein deficiency.

These observations inject a note of caution, long overdue, in regard to the alleged relation between hunger, famine, and infection. In recent years protein nutrition, in particular, has acquired considerable prominence in this regard. The experimental studies of P. R. Cannon (*Advances in Protein Chemistry* **2,** *135 (1945)*) suggest that there is an increased susceptibility or a decreased resistance to infectious disease in the face of protein inanition. Other investigators, as well as several clinical observers, have offered evidence to support this hypothesis. In general, however, laboratory studies pertaining to response to infection associated with protein deficiency have measured phagocytic indices, humoral antibody titers produced by a nonpathogenic antigen, or a lethal end point produced by a virulent, viable pathogen. There have been few concomitant studies of variations in specific physiologic responses to diet and infection. Since under any imposed condition the response of an organism to infection can be expected to be characterized by many adaptive phenomena such studies are desirable before the implied importance of nutritional deficiency in relation to response to infection can be accepted as significant from a clinical standpoint.

Metcoff *et al.* (*loc. cit.*) point out that the simple statement that protein deficiency leads to inadequate response to infection is an oversimplification of a many-faceted phenomenon. An appropriate experimental procedure involving the single variable of protein deficiency, they say, has not yet been reported. The genetic status of the host and pathogen, host factors initiating antibacterial immunity, the source of protein, protein deficiency conditioning other nutritional inadequacies as, for example, that of niacin, and numerous other fundamental variables must be simultaneously controlled before conclusions can be drawn. The importance of endocrine-protein metabolic interrelations and coincident nitrogen balance, liver protein, and protein synthesis studies should be helpful in interpreting the place of protein in infection response. Their studies represent an attempt to correlate a few of the related phenomena. They are careful to point out that the data of their study are not strickly comparable to those of others in which such absolute measures of experimental infection with a virulent pathogen as noninfection or death—complete resistance or complete susceptibility—are specific unequivocal end points.

ASCORBIC AND DEHYDROASCORBIC ACID ANALYSES

The basic 2,6 dichlorophenol indophenol method for estimating ascorbic acid has been compared with the biologic assay method sufficiently to indicate its accuracy for the estimation of biologically active ascorbic acid in many foods. On the other hand, it was recognized that the measurement of dehydroascorbic acid by hydrogen sulfide reduction and subsequent dye analysis was subject to interference by a variety of other

substances which might also undergo reduction. Thus the dinitrophenylhydrazine method of J. H. Roe and C. A. Kuether, which in addition to measuring ascorbic acid appeared to provide a more specific dehydroascorbic acid determination, was of special interest and was widely adopted. Nevertheless there has not been good agreement between the two methods of ascorbic acid analyses. It has been shown (see *Nutrition Reviews* **4,** *148 (1946)*) that the "dehydroascorbic acid" apparently present in aerated orange juice as measured by the dinitrophenylhydrazine procedure was not biologically active, and also that diketogulonic acid, a known decomposition product of dehydroascorbic acid and not biologically active, reacts with dinitrophenylhydrazine.

The report of L. P. Guild, E. E. Lockhart, and R. S. Harris (*Science* **107,** *226 (1948)*) is of interest in this connection, in that it points out the discrepancy between the dye (Bessey) and dinitrophenylhydrazine (Roe) methods for dehydroascorbic acid in pure solutions. In this study these investigators have incidentally confirmed the claim of J. D. Ponting (*Ind. Eng. Chem.* (*Anal. Ed.*) **15,** *389 (1943)*) for the superiority of oxalic acid over metaphosphoric acid for the preservation of reduced ascorbic acid, at least in pure solutions. In the study of reduced ascorbic acid solutions, the Bessey and Roe methods compared closely, but in dehydroascorbic acid solutions, prepared either by charcoal or iodine oxidation, a great discrepancy developed between the results of the two analyses as the solutions were stored. Reduction by hydrogen sulfide followed by dye estimation showed a rapid decrease in dehydroascorbic acid, while the Roe method showed small or insignificant decreases. From the known chemistry of dehydroascorbic acid and from previous work, the investigators were forced to conclude that the Roe method measured degradation products of dehydroascorbic acid that are not biologically active.

From the standpoint of food analysis it is thus questionable whether either method is entirely suitable for the determination of dehydroascorbic acid. Actually the measurement of dehydroascorbic acid in foods has a limited and doubtful value, at least if the food must be cooked before consumption, since this substance is readily destroyed by such treatment. The results of Guild, Lockhart, and Harris demonstrate, however, that the total ascorbic acid as determined by the Roe procedure may be in serious error if significant amounts of "dehydroascorbic acid" are present in the sample analyzed. In the latter event the determination of reduced ascorbic acid by this method must depend on the difference between the "direct" and "after oxidation" values.

The investigators have made the interesting suggestion that the Roe method for total ascorbic acid, because it does include decomposition products of dehydroascorbic acid, may very usefully measure the original ascorbic acid content of a food sample which has been stored or transported with unavoidable loss of the original reduced ascorbic acid content.

INHIBITION OF ACID PRODUCTION BY PRODUCTS OF PROTEIN DECOMPOSITION

The possible inverse relation between dental caries and periodontal disease in malnourished persons has been the subject of a series of investigations by the nutrition investigators at Birmingham, Alabama (*Nutrition Reviews* **5,** *178 (1947)*; **6,** *155 (1948)*).

Since M. Berg, D. Y. Burrill, and L. S. Fosdick (*J. Dental Research* **26,** *67 (1947)*) have demonstrated a correlation between indole production in the oral cavity with the severity of periodontal disease, S. Dreizen and T. D. Spies (*Ibid.* **26,** *409*

(*1947*)) were interested in determining whether indole, indole-3-acetic acid, skatole, or DL-tryptophan would inhibit acid production in human saliva *in vitro* and whether these compounds would inhibit growth and acid production by a pure culture of a specific organism, Lactobacillus acidophilus (Hadley). Fifty-one patients were selected for the study on the basis of these criteria: the presence of ten or more natural occluding teeth, and a history of caries susceptibility as determined from a clinical examination and past results of laboratory tests for dental caries activity. Under supervision, each of these patients collected a specimen of 50 ml. of paraffin-stimulated saliva on which was made a Lactobacillus count, a buffer capacity determination, and the chemical test for caries susceptibility based on the increase in soluble calcium occurring during incubation (L. S. Fosdick, H. L. Hansen, and C. Epple, *J. Am. Dental Assn.* **24,** *1275 (1937)*). One aliquot of the saliva was incubated with dextrose and tricalcium phosphate for four hours in a water bath at 37°C. Another aliquot was incubated under identical conditions with either 7.5 or 10 mg. of indole in addition to dextrose and tricalcium phosphate; a third aliquot was incubated with indole-3-acetic acid. In aliquots of saliva from 10 other patients, the effect of 2 to 10 mg. of skatole per tube was tested. After incubation under identical conditions, the pH, acid production, and the content of soluble calcium of all samples were determined. The differences in values for soluble calcium before and after incubation were used as a possible index of potential dental caries activity (Fosdick *et al., loc. cit.*). In most of the saliva samples, indole, indole-3-acetic acid, and skatole completely inhibited acid production and did not permit increases in the content of soluble calcium by the microorganisms which were present. In the remainder of the samples, acid production was partially inhibited and only small increases of soluble calcium were observed.

To determine the effect of indole, indole-3-acetic acid, and DL-tryptophan on the growth and acid production of L. acidophilus, a pure culture was incubated with yeast extract-dextrose broth both without and with indole, indole-3-acetic acid, or DL-tryptophan in amounts varying from 1.0 to 7.5 mg. per 10 ml. of broth. All tubes were incubated at 37°C. for seventy-two hours; growth and acid production were determined every twenty-four hours. Growth was determined by centrifuging culture aliquots and measuring the packed cell volume; acid production was measured by titration with tenth-normal sodium hydroxide. A second series of cultures was prepared to test the effect of DL-tryptophan on the activity of indole and indole-3-acetic acid. At the end of twenty-four hours, there was no growth or acid production in the tubes to which indole was added in quantities ranging from 2.5 to 7.5 mg. At the end of forty-eight hours, the inhibitory effect was only seen in tubes containing 4.0 to 7.5 mg. and at the end of seventy-two hours, only in tubes containing 6.0 to 7.5 mg. Indole-3-acetic acid inhibited growth and acid production also but to a much lesser extent at all concentrations and incubation periods than was observed in the comparable tubes which contained indole. When DL-tryptophan was added alone, it did not inhibit either growth or acid production. When DL-tryptophan was added to tubes in the presence of indole, the inhibitory effects of the indole were augmented. No increase in the lesser inhibitory effects of indole-3-acetic acid resulted when DL-tryptophan was added.

The actual role that the above inhibitors for acid production and growth of a pure culture of L. acidophilus and of acid production in samples of incubated saliva may have played in the oral cavities of the various groups of malnourished individuals studied by Dreizen *et al.* cannot be evaluated at the present time. An interesting experiment

with caries-susceptible laboratory animals could be performed by the inclusion of various amounts of these inhibitors in caries-producing diets. The use of such inhibitors in animal experiments may be greatly limited by their extremely disagreeable odors and probably would be even more limited in their human application. For a complete knowledge of the causes and control of tooth decay, every positive indication from *in vitro* studies should be carefully followed and evaluated in suitable animal experiments. Under these circumstances, practical knowledge of the various biochemical reactions involved in the carious process can be obtained and those most suitable and most easily applied tested in human experiments.

ABNORMAL METABOLITES IN ASCORBIC ACID DEFICIENCY

In the normal metabolism of the amino acids, phenylalanine and tyrosine, it is believed that hydroxy and keto derivatives, as well as substances having a quinone structure, are intermediates. A well recognized intermediate is homogentisic acid (2,5 dihydroxyphenylacetic acid), and this is the intermediate known to accumulate in alkaptonuria, in which metabolic disease it would appear that further metabolism (by opening of the benzene ring) is prevented. The work of R. R. Sealock and co-workers (see *Nutrition Reviews* **5,** *173, 293 (1947)*) indicates that ascorbic acid is involved in some stage of the metabolism of phenylalanine and tyrosine, probably prior to the homogentisic acid stage. Among the substances accumulating in ascorbic acid deficiency and excreted in the urine, according to E. H. Fishberg (*Proc. Soc. Exp. Biol. Med.* **56,** *24 (1944)*) is an oxidizing agent which can oxidize hemoglobin to methemoglobin. Related to this finding is the chemical picture in "autotoxic enterogenous cyanosis" in which the urine can also oxidize hemoglobin, and in which the same substance circulating in the blood stream appears to be the cause of methemoglobin formation and intense cyanosis.

Fishberg (*J. Biol. Chem.* **172,** *155 (1948)*) has identified the substance responsible for methemoglobin formation in the urine of a cyanotic patient with the above syndrome. It proved to be benzoquinone-acetic acid, the oxidized form of homogentisic acid. As much as 0.5 g. of this substance was excreted by the patient in twenty-four hours. Of particular interest was the report that a scorbutic subject as well as scorbutic guinea pigs also excreted a substance with apparently identical chemical and physical properties. A series of seven tests were described for benzoquinone-acetic acid, and these were all positive in the urine and urine distillate from the cyanotic and the scorbutic patient.

Crystalline benzoquinone-acetic acid was prepared from the urine of the cyanotic patient, and positively identified by preparation of the dimethyl ether of homogentisic acid. The oxidation-reduction potential of the quinone in urine was measured, and its high potential readily explains the oxidation of hemoglobin to methemoglobin. The ultraviolet spectrum of benzoquinone-acetic acid was given, and this was the same as the material separated from the urine in a case of rheumatic fever, in which condition, like scurvy, there is a diminished excretion of ascorbic acid.

A quantitative method for the determination of water soluble p-quinones was adopted which involved the liberation of iodine from potassium iodide, and titration with sodium thiosulfate. With this method the daily excretion of benzoquinone-acetic acid was followed in the cyanotic patient and correlated with ascorbic acid excretion and the oxygen capacity of the blood. During the first few days large quantities of benzo-

quinone-acetic acid were excreted, very little ascorbic acid was excreted, and the blood oxygen capacity was only 16 volumes per cent. It was estimated that 20 per cent methemoglobin was present. During a successive five day period, the benzoquinone-acetic acid excretion became zero, while ascorbic acid excretion was markedly increased, and the oxygen capacity also increased. Further periods of study showed the same inverse relation between benzoquinone-acetic acid and ascorbic acid excretion. On the thirtieth day of the study the patient was given 500 mg. of ascorbic acid by mouth and 500 mg. intravenously daily. The excretion of quinone promptly ceased and the excretion of ascorbic acid reached 500 mg. The oxygen capacity reached 19 to 20 volumes per cent, and the methemoglobinemia and cyanosis completely disappeared.

It was not the object of Fishberg's paper to discuss the biochemical defect in "autotoxic enterogenous cyanosis," whether for example it is to be considered primarily as a disturbance of ascorbic acid metabolism in which quinone formation is a sequel, or whether there is primarily an abnormal formation of the quinone and resulting oxidation of ascorbic acid. In scurvy, at least, the formation of the quinone must be due to lack of the biochemical function of ascorbic acid in tyrosine metabolism. When the benzoquinone-acetic acid is produced in exceptional quantities, it appears in the urine and a large part of the hemoglobin may be converted to methemoglobin.

FAMINE EDEMA

The logical explanation that famine edema is the result of lowered plasma protein concentrations due to protein deficiency has been the generally accepted doctrine for many years. Results obtained by A. Keys and associates (*Nutrition Reviews* **5**, *129 (1947)*) and by others directly in famine areas in Europe are apparently at variance with this elegant and long taught explanation. Such data show various degrees of proteinemia with or without little correlation with the presence or extent of edema and it seems clear that factors other than the plasma protein level are involved.

P. Govaerts (*Compt. rend. Soc. biol.* **89**, *678 (1923)*) was one of the first to show, by direct osmotic pressure measurements on serum, the importance of the plasma proteins in the development of edema in cardiac and nephritic patients. He has now summarized studies done in Brussels in 1941 to 1942 ("VII^{e} Congrès de Chimie Biologique, Liège, 3–6 Octobre, 1946," *p. 23, Masson, Paris (1947)*) during a famine period. The patients were said to have a caloric intake not exceeding 1500 calories per day and a protein intake of 25 g. per day. No meat or fish were available. The following measurements were made: osmotic pressure of the serum by means of an osmometer, total plasma proteins either by a refractometric method or by micro-Kjeldahl determination, albumin-globulin ratio by the Howe method of sodium sulfate precipitation, venous pressure, circulation time, basal metabolism, and cardiac output. All determinations were not made on all patients but rather complete data are presented for 48 patients showing various degrees of edema.

Serum proteins were below normal in the majority of the patients ranging from 3.6 to 8.8 per cent with most between 5.4 and 7.2 per cent. Osmotic pressure of the serums was also low, ranging from 12 to 35 cm. of water compared with 35 to 40 cm. in normal subjects. However, whereas the mean protein concentration was found to be 28 per cent below normal the mean osmotic pressure was decreased by 41 per cent. In previous work Govaerts found the osmotic

pressure in centimeters of water to be equal to 7.54 times grams of albumin plus 1.95 g. of globulin utilizing the method of Howe for the determination of the two protein fractions. The present work, said to be done in identical fashion, yielded calculated values considerably above the actually determined osmotic pressure in the majority of the cases. The conclusion is thus drawn that not only are the plasma proteins somewhat low in most instances of famine edema, but that the osmotic pressure per gram of protein is also decreased below normal values. This rather startling conclusion will undoubtedly meet with considerable skepticism but the implications, should it be confirmed, are important. As stated by Govaerts, they indicate either a change in the behavior of the proteins during sodium sulfate precipitation or a change in structure of the proteins. M. Florkin and G. Duchateau (*Bull. Acad. roy. Méd. Belg.* **8,** *573 (1943);* **9,** *91 (1944)*) are reported as having reached similar conclusions.

The effective osmotic pressure of the blood could be reduced by the presence of large amounts of protein in the interstitial fluid. Such was not found and therefore changes in capillary permeability are considered unlikely as a cause of edema in starvation.

The basal metabolism of the starvation patients was well below normal in most cases. Data upon 39 patients show 30 between −15 and −60. Similarly, the pulse rate was between 38 and 64 in most cases. The venous pressure was slightly above normal in only 11 out of 47 patients. A composite electrocardiogram shows the voltage of all the complexes to be decreased and the QT interval considerably prolonged. The circulation time of normal subjects by the method used is reported as between 12 and 17 seconds, while 42 of 48 of these patients had a circulation time over 17 seconds and, as expected, the difference in the oxygenation of arterial and venous blood was considerably greater than normal. Cardiac output in 10 of the patients showed a mean value of 1.13 liters per minute compared with a normal value of 4 liters per minute.

Upon admittance to the hospital the diet was not changed. However, with bed rest a diuresis with disappearance of edema usually was noted within twenty-four hours. This was accompanied by considerable improvement in all of the measurements made on the circulation, the basal metabolism improved moderately, but the changes in the plasma proteins were insignificant.

Govaerts concludes that famine edema like other edemas results from a failure in the equilibrium between the osmotic pressure of the plasma and the hydrostatic pressure of the tissues. With marked loss in weight the pressure in the tissues tending to return fluid to the capillaries probably falls. At the same time, there is a decrease in the osmotic pressure over and above that which can be accounted for by the fall in plasma proteins. Finally, the sluggish circulation is probably an important factor since edema usually occurs in the lower extremities and disappears with bed rest.

THE SLR FACTOR (RHIZOPTERIN) AND FORMYL FOLIC ACID

The rapid developments in the chemistry of the folic acids during the past two years have made possible a precise classification of several of the members of this group. Of the two remaining members whose structures were unreported (spinach folic acid and the SLR (Streptococcus lactis R) factor) the latter has recently been described in detail, and as a result new information has been gained regarding the functional role of folic acid.

Announcement of the discovery of the SLR factor was made in a brief note (J. C. Keresztesy, E. L. Rickes, and J. L. Stokes,

Science **97,** *465* (*1943*)), in which it was demonstrated that a factor existed which was active for Streptococcus lactis R (S. faecalis) but was inactive for Lactobacillus casei. Later, Stokes, Keresztesy, and J. W. Foster (*Ibid.* **100,** *522* (*1944*); *Nutrition Reviews* **3,** *165* (*1945*)) converted the SLR factor into an L. casei factor by incubation with resting cells of any of several streptococci, including S. faecalis.

Although the SLR factor had been obtained in pure form at the time of these reports, no description of the isolation was furnished, nor was the source of the factor disclosed. These and other pertinent data have now appeared (Rickes, L. Chaiet, and Keresztesy, *J. Am. Chem. Soc.* **69,** *2749* (*1947*)). The SLR factor was derived from the liquors remaining after fumaric acid fermentation by Rhizopus nigricans. Subsequent investigations revealed the pterin-like structure of the compound, and the factor was therefore called rhizopterin.

The authors report that a two hundred thousand fold concentration was effected to obtain crystalline rhizopterin. This was accomplished by a series of absorptions and elutions with final crystallization as the free acid or ammonium salt. This material in quantity of 0.000034 microgram per milliliter of medium produced half maximum growth of S. faecalis.

Acid or alkaline hydrolysis of rhizopterin produced formic acid and pteroic acid. The latter is about one-tenth as active as rhizopterin for the test organism (Rickes, N. R. Trenner, J. B. Conn, and Keresztesy, *J. Am. Chem. Soc.* **69,** *2751* (*1947*)). No glutamic acid was obtained. In another series of studies, workers at the Merck laboratories (D. E. Wolf *et al.*, *Ibid.* **69,** *2753* (*1947*)) showed that the rhizopterin structure is essentially that of pteroic acid, i.e., a "pteridyl" para-aminobenzoic acid, except that the para-amino group is formylated (—N(CHO)CH_2—). The glutamic acid residues which characterize most of the folic acid group are absent in rhizopterin. It seems likely that in the biosynthesis by microorganisms referred to above, a conjugation of rhizopterin with glutamic acid was effected, thus producing a structure utilizable by L. casei. Since this synthesis takes place to an extent much beyond the requirements of the organisms for growth, it may be inferred that the activity of rhizopterin is due to its conversion to a glutamate. Rhizopterin, and probably pteroic acid as well, might thus be considered precursors of folic acid.

The formyl group in this compound appears to have special significance in relation to studies of Shive and co-workers (W. Shive *et al.*, *J. Am. Chem. Soc.* **69,** *725* (*1947*); M. Gordon, J. M. Ravel, R. E. Eakin, and W. Shive, *Ibid.* **70,** *878* (*1948*)) on an analogous formyl derivative of pteroylglutamicacid. In their first note, these latter workers found that when sulfanilamide was used to prevent growth of Escherichia coli, 4(5)-amino-5(4)-imidazole carboxamide accumulated in the medium. This imidazole differs from that of a purine skeleton by a single carbon atom, and the authors felt that para-aminobenzoic acid or a compound formed from it served to bring about the conversion to a purine. After the structure of rhizopterin was announced, they prepared formyl folic acid, which is related to pteroylglutamic acid as rhizopterin is to pteroic acid. Formyl folic acid was thirty times as effective as PGA in reversing inhibition by methyl folic acid in S. faecalis. Moreover, rhizopterin was two or three times as effective for this purpose as PGA. Finally, the toxicity of methyl folic acid was increased several fold after heating with formic acid for two hours. These observations supply additional evidences of a role of the formyl group in the formyl folic acid molecule, and suggest that the vitamin is a formylating agent. Recalling experiments with E. coli, the authors suggest that formyl folic acid may function to incorporate the single carbon unit into the imidazole precursor of purines.

These concepts may be related to the

study of E. L. R. Stokstad, J. Pierce, T. H. Jukes, and A. L. Franklin (*Federation Proceedings* **7,** *193* (*1948*)) indicating that PGA is necessary for the synthesis of the pyrimidines, thymine and adenine, by S. faecalis R.

Evidence bearing on the possibility of similar relations in man may be summarized as follows: the three pteroylglutamates (mono-, tri-, and hepta) are hematopoietically active (*Nutrition Reviews* **6,** *131* (*1948*)), pteroic acid is inactive (T. D. Spies and R. E. Stone, *South. Med. J.* **40,** *46* (*1947*); E. Jones, H. F. Warden, and W. J. Darby, *Am. J. Med.* **3,** *506* (*1947*)); and the magnesium salt of both formylpteroic acid (rhizopterin) and formylpteroylglutamic acid failed to show hematopoietic activity (Spies *et al.*, *Blood* **3,** *121* (*1948*)). Large amounts of 5-methyl uracil (thymine) have an hematopoietic effect in macrocytic anemias (W. B. Frommeyer and Spies, *Am. J. Med. Sci.* **213,** *135* (*1947*)). It appears, therefore, that only the glutamates and thymine are active in man, but that the formyl derivatives are not available to man. Rickes, Chaiet, and Keresztesy (*loc. cit.*) note that rhizopterin in doses up to fifty times the effective dose of PGA is ineffective in improving folic acid deficiencies in chicks or rats. The available evidence, therefore, indicates that the possible role of these formyl derivatives in pyrimidine and purine synthesis is not the limiting metabolic role in higher animal species.

DYNAMICS OF PROTEIN METABOLISM

Although T. B. Osborne, L. B. Mendel, and E. L. Ferry stated long ago (*J. Biol. Chem.* **37,** *223* (*1919*)) that economy in nutrition during growth depends upon the correct adjustment between the proportions of protein and total energy supplied, information regarding the requirements for reconstruction of lost tissue has been limited. Because optimal nutrition remains a major problem of medicine and surgery despite the accumulating knowledge concerning vitamins, amino acids, and other essential dietary factors, E. P. Benditt *et al.* (*J. Lab. Clin. Med.* **33,** *257* (*1948*)) have sought to determine the interrelation between the level of protein intake, the energy intake, and the utilization of dietary protein under controlled conditions.

The basic plan of their study was to determine the rate of utilization of protein for tissue fabrication by protein depleted animals under three different circumstances: (1) varying caloric intake with constant protein intake; (2) varying protein intake with constant caloric intake; and (3) simultaneous variation of caloric and protein intakes.

Young adult male albino rats were used. At the start of the depletion period their weights averaged 210 g. For two and one-half months they were fed a low protein diet which contained adequate calories, vitamins, minerals, and other dietary essentials. At the end of the depletion period the average weight of the animals was 154 g. Groups of 5 or 6 animals were then selected to be fed each of the experimental diets. The animals were chosen so that each group was comparable with respect to initial weight, weight loss, serum protein concentration, and hemoglobin concentration.

For the protein of the diet a half and half mixture of bovine lactalbumin and vitamin free casein was used. The basic composition of the diets was the same in all three experiments. Diets were offered in weighed amounts each day. Diet consumption was measured daily and the experimental feeding period was fourteen days. At the end of the test periods the animals were killed and the liver, heart, kidneys, and approximately 40 per cent of the blood were removed. The gastrointestinal tract was stripped of its contents. The carcass with the residual contents including the gastrointestinal tract was

weighed and analyzed for water, fat, protein, and ash. Protein gains were measured by subtracting the mean quantity of protein found in the carcasses of a control group of depleted animals from the quantity determined in each of the repletion groups. The value thus obtained included the quantity of protein lost by the control group; that is, the wear and tear protein loss. This quantity was determined and subtracted from the apparent gains of the animals in the repletion groups to obtain a measure of protein fabrication over and above that which merely replaced the wear and tear losses. To include all or practically all of the protein fabricated in the experimental interval, the increase in the liver protein was added to that in the carcass protein.

At the lowest level of caloric intake (560 calories per square meter per day), with a constant protein intake of 1.56 g. per day, protein utilization was poor (0.3 g. per kilogram per day). With increasing caloric intake, protein utilization rose until a maximum was reached at an intake level of 1240 calories per square meter per day. Beyond this point there was no additional utilization of protein despite the caloric intake being increased up to 1840 calories per square meter per day.

Varying the protein intake at a constant caloric intake (1630 calories per square meter per day) was associated with increased protein utilization as the protein intake was raised from 2.8 to 14.8 g. per kilogram per day. Beyond 6 g. per kilogram per day, however, the rate of protein utilization became progressively less.

When increasing quantities of the same diet were fed to the depleted animals with thus, a constant ratio of protein to energy in the diet, the rate of protein utilization increased with increasing diet intake. The data indicated that the limitation in the rate of protein utilization was not the summation of the effects of the restriction of both protein and energy intakes. The restriction of utilization at any point was no greater than the maximal restriction induced by the restriction of the protein component alone.

The quantity of fat deposited at a constant protein intake and increasing caloric intakes was approximately proportional to the caloric intake over the range studied.

The observations of Benditt *et al.* on protein depleted animals are in agreement with those reported heretofore on patients and growing animals. There appears to be a restriction in the utilization of a constant quantity of protein when the caloric intake falls below a critical value of about 1240 calories per square meter per day.

The significance of this critical caloric value is explained as follows. From carcass analysis data and food intake energy balances, it was calculated that the animals stored 323 calories per square meter per day as new tissue. The difference between the total intake and the energy appearing as new tissue represents the quantity consumed for energy and wasted. Therefore, the animals must have consumed and wasted a total of 917 calories per square meter per day. Since animals receiving 560 calories per square meter per day were able just to maintain themselves during a fourteen day period, this figure was taken to be an approximation of the minimal maintenance energy requirements. The difference between the total energy consumed (917 calories) and the energy used for maintenance (560 calories) represents the energy used to construct new tissue plus waste energy, in this case 357 calories per square meter per day. The quantity, 1240 calories per square meter per day, therefore appears to represent the energy allowance which will supply the maintenance needs of the animals with sufficient excess to allow for maximal utilization of the ingested protein for tissue synthesis. At caloric intakes below the critical level the animal must resort to burning protein for purely energy purposes. As a consequence, the restriction of caloric intake below the

critical point has the same net effect as a restriction of the protein intake.

In another report (E. P. Benditt, R. L. Woolridge, and R. Stepto, *J. Lab. Clin. Med.* **33,** *269 (1948)*), studies are described in which protein intake ranges commonly used and feasible in human feeding were employed. The diet given these rats offered approximately 0, 1, 2, 3, and 4 g. of protein per kilogram body weight per day. With protein intakes ranging from 0.5 to 4.0 g. per kilogram of body weight per day, utilization of protein for tissue synthesis was also proportional to the protein intake. There was good agreement with published data on normal individuals as well as with observations on cancer patients and on patients convalescing from gastrectomies for peptic ulcers fed through oral jejunal tubes.

The observations of Benditt and his associates are not in accord with those of A. Keys (*J. Am. Dietet. Assn.* **22,** *582 (1947)*) who stated that, in relief feeding, calories are of overwhelming importance. Benditt *et al.* conclude that while caloric intake is a factor, it is not the only factor. Moreover, with an adequate caloric intake the level of protein intake and the quality of the protein become the limiting factors. Protein intake levels of 2 to 4 g. per kilogram per day are demanded for the rapid rehabilitation of a depleted person. Some fat gain should also be allowed in rehabilitation of depleted individuals for the purpose of building energy reserves. Benditt and his coworkers suggest that the rational procedure is to feed protein as a source of building material and compute the energy value of the diet, independent of its protein content, on the basis of its carbohydrate and fat content. It is suggested that for an average sized man (surface area 1.7 square meters), afebrile and at bed rest, 2600 calories per day or 1500 calories per square meter per day are needed.

The two papers by Benditt *et al.* provide two of the first convincing summaries to show the value of high protein intakes during rehabilitation following protein depletion, and animal experiments and clinical experience now begin to show some semblance of order. However, the last word on the relation between caloric and protein intakes has not yet been written. It should be noted that in the rat experiments providing a constant protein intake with variable caloric intakes the level of protein fed was not high, 1.56 g. per day, and at maximum utilization the diet provided about 15 per cent of protein. The other data show, however, that much better protein utilization is obtained upon a diet providing only 11 per cent of protein when food intake is high enough to allow an intake of 1.82 g. of protein per day. With additional experiments it should be possible to show the level of protein intake which will yield maximum efficiency for various levels of caloric intake. In evaluating the rat data it must also be remembered that these animals were very severely depleted, having lost approximately 25 per cent of their body weight, and that they have marvelous recuperative power since they regain this weight within two weeks, or less, after receiving a good diet. Under such conditions the ability to use protein is enormous.

ASCORBIC ACID AND INDUSTRIAL POISONS

The possible role of vitamins in detoxication processes has been studied frequently. The practical importance of decisive knowledge on any specific protective action of a vitamin against an industrial toxin is obvious. Benzene is a widely employed potentially toxic industrial solvent. Signs of benzene poisoning include petechial hemorrhages, a clinical sign also seen in scurvy. The hemorrhage in the former condition,

however, is on the basis of thrombocytopenia in contrast to the deficiency disease. Another superficial relation between ascorbic acid and benzene is the role of ascorbic acid in the breakdown of the amino acids, tyrosine and phenylalanine, both of which contain benzene rings. These relations have often given rise to the suggestion that ascorbic acid may exert a specific protective action against benzene poisoning (see B. Ekman, *Acta physiol. Scandinav.* (*Suppl.*) **22,** *12* (*1944*)).

An attempt at a direct approach to the question of a relation between benzene poisoning in man and ascorbic acid metabolism has appeared (S. Forssman and K. O. Frykholm, *Acta med. Scandinav.* **128,** *256* (*1947*)). This paper reports studies of the urinary concentration of and tests for "saturation" of ascorbic acid on two groups of workers who were exposed to benzene, and on a control group of workers who received no exposure. Random specimens of morning urine (not twenty-four hour samples) were analyzed titrimetrically for ascorbic acid, gravimetrically for total and inorganic sulfate (difference = ester sulfate), as well as for other products of benzene degradation. The "saturation tests" were carried out by oral administration of 600 mg. of ascorbic acid on the first day, and 300 mg. daily thereafter until two urinary concentrations of 7 mg. per cent were obtained. One-hundred and two rotogravure workers were studied; these were approximately equally divided into exposed and control (non-exposed) groups in each of two plants. The benzene concentration of the room air in which the exposed groups worked averaged 1.1 and 0.41 mg. per liter, respectively, in Plants I and II.

The average urinary ethereal sulfate concentration paralleled the average exposure to benzene and was not affected by the administration of ascorbic acid. Prior to administration of ascorbic acid the findings in Plant I compared as follows for the exposed and control groups: average concentration of urinary ascorbic acid, 2.2 mg. per cent and 1.7 mg. per cent. The average results of the saturation studies in the same groups were respectively: number of days of dosing before saturation was obtained, 8.4 and 6.7; maximum ascorbic acid concentration of urine, 14.2 and 23.1 mg. per cent. The variation between individuals was great so that interpretation of these means should be cautious. A statistically significantly larger number of exposed workers were judged to be unsaturated in each of the plants.

The workers concluded that ". . . exposure to benzene produces an increased requirement of vitamin C and that an extra supply of vitamin C gives increased resistance to the effects of benzene vapors." A more valid conclusion would appear to be that persons exposed to vapors of benzene may have lower ascorbic acid stores than workers who are not so exposed. No evidence is here presented that benzene increases the requirement. The observed effect of exposure on urinary level could merely reflect differences in dietary intake which, in turn, might have been conditioned by exposure to the benzene. An illustration of the need for more precise thinking in arriving at a conclusion from such data as these is provided by the excellent report of E. E. Evans, W. D. Norwood, R. A. Kehoe, and W. Machle (*J. Am. Med. Assn.* **121,** *501* (*1943*)) in which they studied ascorbic acid in relation to another industrial toxin, lead.

These investigators studied the effects of ascorbic acid status on 72 workers in a tetraethyl lead plant. The group of workers had been under careful observation for years, and a background of data was available on them as to lead excretion in the urine and stool, symptoms, illnesses, and other pertinent medical observations. Preliminary observations of the ascorbic acid nutrition of the "exposed" group as compared with a similar control group which was not exposed to lead may be summarized as follows: A higher percentage of the exposed group had low plasma ascorbic acid levels, low twenty-

four hour urinary excretion of ascorbic acid, and were judged as unsaturated on the basis of a urinary saturation test as well as a test which measures the rise in plasma ascorbic acid following a standard injection of the vitamin (L. Kajdi, J. Light, and C. Kajdi, *J. Pediatrics* **15,** *197 (1939)*). These tests were not made on all of the subjects, but the results of each type of measure revealed corresponding differences between the groups.

Thirty-six of the "exposed" men were given 100 mg. of ascorbic acid daily by mouth for one year. The other 36 "exposed" workers served as unsupplemented controls. Identical data were kept on the supplemented and unsupplemented exposed individuals. These included symptomatology, physical findings (especially blood pressure), hematologic studies, and concentration of lead in blood, urine, and stools. No effect of the supplementation could be discerned on lead concentration in blood, urine, or stool, hematologic findings, on physical condition of the workers, or on the number and severity of complaints. These conclusions were based upon a comparison of the data from the supplemented group with those from the unsupplemented exposed group, as well as a comparison of the supplemented group with the accumulated background of observations previously made on them. The investigators concluded that no reason was found for recommending the use of ascorbic acid to minimize the effects of lead absorption.

Here, then, is an example of a critical therapeutic testing of an initial suggestive correlation between factors. The correlation had been consistent with a hypothesis that ascorbic acid might be expected to minimize the effects of the industrial poison. The test did not bear out this expectation. Such experiences emphasize that correlation does not signify causation. The implication of the observations in this study of lead poisoning for the one reviewed on benzene toxicity is obvious.

It is to be hoped that investigations will continue of seemingly promising leads concerning the relation between vitamins and industrial poisons, such as ascorbic acid and benzene. The problem should be clearly defined, however, and the conclusions soundly based upon the facts.

DIETARY FAT AND THE GENESIS OF EXPERIMENTAL HEPATOMAS

There are many experiments on record indicating that the development of tumors in laboratory animals may be accelerated or retarded by variations in the diet fed. The effects in experimental animals of abnormal diets upon chemical carcinogenesis has led to the accumulation of data, much of which is difficult to interpret, and much apparently contradictory. Thus diets low in calories retard the development of various tumors, while diets high in riboflavin are particularly effective against the formation of liver tumors in rats fed azo dyes. The work from the Wisconsin laboratories has been reviewed by H. P. Rusch and C. A. Baumann (*Nutrition Reviews* **4,** *353 (1946)*). Specifically, they found that tumor formation in rats fed diets containing small quantities of the dye para-dimethylaminoazobenzene is inhibited by feeding riboflavin, egg white, or hydrogenated coconut oil. Further work by the Wisconsin group has served to clarify partially the inhibiting effect of dietary coconut oil and of riboflavin upon carcinogenesis (J. A. Miller, B. E. Kline, Rusch, and Baumann, *Cancer Research* **4,** *153, 756 (1944)*). Corn oil did not inhibit carcinogenesis. The different actions of hydrogenated coconut oil and corn oil did not appear to rest on dissimilarities in antioxidant properties, nor upon the presence or absence of linoleic acid. These studies were made with animals on a

synthetic diet. On a highly carcinogenic diet containing crude casein and a rice bran extract, however, a high tumor incidence was observed with both corn oil and hydrogenated coconut oil.

Experiments by Kline, J. A. Miller, Rusch, and Baumann (*Cancer Research* **6,** *1* (*1946*)) were designed to determine whether the fatty acids themselves were responsible for the differing action of the coconut and corn oils. Each of these fats was hydrolyzed and the fatty acids fed with glycerol at the same dietary level as in previous experiments. Another group of rats was fed a diet in which lauric acid, the chief fatty acid of coconut oil, replaced the fat. As controls, one group of rats was fed on the standard corn oil ration and one group on a low fat diet. In addition, olive oil and oleic acid were fed at the same dietary level in two other groups of rats. Average daily food consumption and weight gain during four of the six month study period was about the same for all groups. The incidence of tumors in the groups fed corn oil was high, as it was in the group fed the fatty acids of corn oil. In contrast, both the group fed the fatty acids from the coconut oil and the group fed lauric acid did not develop tumors even after six months. Tumors developed somewhat less readily when olive oil was fed than when the diet contained corn oil, but on oleic acid tumors developed rapidly. Only a few of the rats fed the low fat diet developed tumors. The authors concluded that lauric acid probably accounted for the anticarcinogenic effect of the hydrogenated coconut oil.

In a second paper (*Cancer Research* **6,** *5* (*1946*)) the same authors found that the quantity as well as the type of fat affected carcinogenesis following the feeding of para-dimethylaminoazobenzene. Similar methods and diets were used as in the previous study, except that in addition to a low fat diet and diets containing 5 per cent corn oil and 5 per cent olive oil, three other groups of rats were fed diets containing, respectively, 20 per cent of corn oil, lard, or Crisco. The inhibitory effect of hydrogenated coconut oil and of lauric acid was not studied in this series of experiments. As in the previous study the incidence of tumors was lower in the low fat group than in the groups fed diets containing corn or olive oil. Among the groups receiving the high (20 per cent) fat diets, the corn oil appeared actually to favor carcinogenesis. The food consumption and daily average weight gain in the group fed the high fat diet were much poorer than that of the other group. The groups fed 20 per cent Crisco and lard were similar in weight gain, food consumption, and carcinogenesis to those fed 5 per cent of corn oil. The authors conclude that not only does 5 per cent of corn oil favor carcinogenesis, but 20 per cent is even more effective. However, Crisco and lard were no more effective than 5 per cent corn oil whether they were fed at a 5 per cent or 20 per cent level in the diet.

Further studies on the inhibitory action of hydrogenated coconut oil are presented in three subsequent papers from the same laboratory (J. A. Miller, Kline, and Rusch, *Cancer Research* **6,** *674* (*1946*); J. E. Giese, C. C. Clayton, E. C. Miller, and Baumann, *Ibid.* **6,** *679* (*1946*); E. C. Miller and J. A. Miller, *Ibid.* **7,** *468* (*1947*)).

In one series of experiments small amounts of two detergents were fed in addition to 5 per cent corn oil. These diets were highly anticarcinogenic, having activity equivalent to that of the hydrogenated coconut oil. In another group of rats, 5 per cent of mineral oil was used to replace the corn oil. Here again anticarcinogenesis was observed, but these animals lost rather than gained weight despite a high food consumption, probably from poor absorption due to the mineral oil contained in the diet. Although the mineral oil series was not conclusive because of the weight loss, it was thought that both types of substances might be acting by diminishing absorption of the carcinogen. With this in mind the authors studied the fecal excretion of the para-dimethylaminoazobenzene but found only small

amounts of the dye present. The liver and blood concentrations of the dye were somewhat reduced in those animals fed the protective factors, but whether the reduction was great enough to prevent tumor formation could not be determined from these experiments. Thus, the possibility remained that the inhibitors might exert their action by rendering the carcinogen less available.

To study the latter possibility the authors replaced para-dimethylaminoazobenzene in the diet by one of two other carcinogenic dyes: m′- methyl-p-dimethylaminoazobenzene and o′-methyl-p-dimethylaminoazobenzene. With the incorporation of the more powerful carcinogen, the m′-methyl dye, in the diet, a high incidence of tumors was observed which was neither increased by rice bran extract nor inhibited by hydrogenated coconut oil. There was some inhibition, although not great, by the addition of riboflavin to the diet. When the o′-methyl dye, a carcinogen weaker than para-dimethylaminoazobenzene, was fed there was definite although not great reduction in tumor incidence by feeding of both inhibitors, i.e., hydrogenated coconut oil and riboflavin. Thus, it appears that hydrogenated coconut oil has its greatest inhibitory effect when the carcinogen is para-dimethylaminoazobenzene, that it is less effective against the o′-methyl dye, and without significant inhibitory effect upon the m′-methyl compound. In contrast, the riboflavin exerted an inhibitory action with all these compounds, although not nearly as great against the m′-methyl compound as against the somewhat less powerful carcinogen, para-dimethylaminoazobenzene. The authors believe that riboflavin exerts the most general inhibitory action, suggesting that its effect is upon the carcinogenic reaction itself. The other inhibitory agents, the detergents, mineral oil, and especially the hydrogenated coconut oil, because of their irregular action with different compounds, may be thought of as having their effects upon the carcinogen itself rather than upon the reaction of the carcinogen with the tissue.

A later paper from the Wisconsin laboratories by E. C. Miller and J. A. Miller (*Cancer Research* **7**, *468 (1947)*) deals with the accumulation of an azo dye tightly bound in the livers of rats fed para-dimethylaminoazobenzene. The new dye was bound to both protein and nucleoprotein and the greatest concentration of bound dye in the liver was reached after four to six weeks of feeding. No significant amount of bound dye was found in tissues other than liver, suggesting that the protein-dye compound is important in the pathogenesis of the tumors. This was further borne out by an apparent correlation between the quantity of bound dye in the liver under different circumstances, and the incidence of tumor formation. Thus, the inclusion of large amounts of riboflavin in the diet not only retards carcinogenesis but it also decreases the amount of bound dye found in the liver. The inclusion of riboflavin in the diet appeared also to increase the rate of disappearance of the dye from the liver when dye feeding was stopped. The authors believe that the combination of azo dye with liver protein is a primary step in carcinogenesis by the dye. Experiments to show whether hydrogenated coconut oil, the detergents, and mineral oil fed in the synthetic diet with the carcinogen will reduce the amount of bound carcinogen in the liver will be awaited with interest.

REQUIREMENT OF RATS FOR AN "ANIMAL PROTEIN FACTOR"

Several reports have indicated that consumption by laying hens of rations containing all-plant proteins results in lowered hatchability of the eggs, and a decreased growth rate in chicks which do hatch from such eggs. A nutritional factor which protects against these injurious effects is present in dried cow manure, and in proteins of

animal origin (*Nutrition Reviews* **6**, *71 (1948)*).

A number of papers have reported that a similar factor or factors may be necessary for optimal growth of rats fed rations complete in known nutritional essentials, but deriving their protein wholly from plant sources. One of the most detailed of these is the paper of L. M. Zucker and T. F. Zucker (*Arch. Biochem.* **16**, *115 (1948)*). These authors fed rations well fortified in the known vitamins and in protein. Cottonseed meal was the chief protein source in each of the rations fed, with small amounts supplied by alfalfa leaf meal and gelatin. The ration also contained 2 per cent of a brewers' yeast extract as a source of unidentified vitamins of the "B group." When female breeders were transferred from the stock diet to this basal ration and mated, prompt impregnation, normal gestation, and normal lactation occurred. At weaning (28 days), the young averaged only about 15 per cent less in weight than normal controls. When these animals were continued on the basal ration, however, all grew poorly, and 30 of 66 died, mostly within a few days of weaning. Death probably resulted from hemorrhage in the fundic portion of the stomach. Survivors of this critical period continued to grow slowly, and at 20 weeks were 40 per cent below normal weight for this age. There was a tendency for individual litters either to survive entirely or wholly succumb, a behavior indicative of differences in requirement, storage, or synthesis of the missing factor. The blood of such animals showed high urea values as compared with blood of normal animals with a similar protein intake. The spleen was shrunken, and the kidneys and liver were somewhat enlarged, although they presented a normal histologic appearance. During the period of crisis (i.e., the few days following weaning) a definite leukopenia was evident.

All of these changes in the young were prevented by incorporation of 12.5 per cent of crude casein in the basal ration fed to the mothers. Five to 10 per cent of condensed fish press water ("fish solubles") or 5 per cent of 1:20 liver powder were also wholly effective in preventing the deficiency symptoms. One-half of 1 per cent of liver powder was not quite as effective as 5 per cent. Three different lots of brewers' yeast, in amounts up to 10 per cent of the ration, were ineffective in preventing the deficiency condition. The protective factor present in these animal materials, but lacking from yeast and many plant proteins, has been tentatively named "zoopherin."

As stated above, these deficiency symptoms appear in the young of female rats placed on the basal ration at the time of mating. No deficiency symptoms are seen in the mothers themselves. When weanling rats from mothers on stock ration are put on the deficient ration at 4 weeks of age, some growth retardation occurs, beginning at 6 to 7 weeks of age on the most deficient diets. When given a source of zoopherin, growth is speeded and eventually returns to normal. When no zoopherin is given, and females are continued on the ration through a pregnancy, the deficiency produced in the young is more marked, and some deaths occur during the early days after parturition, before weaning. These may result in part from faulty lactation performance by the mothers.

A number of curative experiments were carried out on the severely deficient surviving rats from litters of dams which had been on the deficient ration from weaning time on. These animals grew very slowly for forty-two to fifty-five days after weaning. At this time, one group was transferred to the stock ration, one was continued on the deficient ration supplemented with 5 per cent liver powder, and one group received 10 per cent of Labco vitamin-free casein, and the deficient ration. The first two groups grew and recovered rapidly, the group receiving 10 per cent of the vitamin-free casein failed to grow. Some of the latter animals were transferred after seventeen days to a supplement of 10 per cent crude casein, whereupon growth picked up sharply.

These experiments demonstrate that the

deficiency is not due to lack of protein, but to lack of a substance associated with animal protein, which can be separated from the latter by appropriate purification procedures. It cannot be identical with known vitamins of the "B group," since it is not supplied even by 10 per cent of brewers' yeast. Folic acid (pteroylglutamic acid) at high levels was ineffective as a supplement to the deficient ration. A concentrate of the chick growth factor of cow manure (*Nutrition Reviews* **6,** *71 (1948)*), however, was highly active in preventing zoopherin deficiency, and it seems possible that the two factors may be identical. Only a few properties of the new dietary factor have been so far described. It is very soluble in water, dilute acid, alkali, and aqueous alcohol, moderately soluble in 95 per cent ethanol, and insoluble in petroleum ether and ethyl ether. The latter properties differentiate it from the lipid-soluble "animal-protein factor" for chicks referred to by G. F. Heuser, L. C. Norris, H. L. Lucas, and G. F. Combs (*Poultry Sci.* **25,** *403 (1946)*) and by E. L. Johnson, C. W. Carrick, R. E. Roberts, and S. M. Hauge (*Ibid.* **21,** *566 (1942)*).

Similar, though less detailed evidence for the existence of a new dietary factor for the rat, the requirement for which becomes particularly evident only under the strain of reproduction or lactation, and which is not identical with the known vitamins but is associated with animal products, has been presented by C. A. Cary and A. M. Hartman (*U. S. Dept. Agr. Yearbook, p. 779 (1947)*) and by J. P. Bowland, M. E. Ensminger, and T. J. Cunha (*Arch. Biochem.* **16,** *257 (1948)*), and others. In addition to the intrinsic interest in the demonstration of a new essential food factor, such work emphasizes that careful and thorough testing over prolonged periods of time and more than a single generation is necessary to prove the adequacy of an experimental diet. Only in this way can one be assured that an animal is not receiving part of its nutrient requirements from maternal stores. Perhaps more than one new nutritional essential remains to be discovered by this approach.

STARVATION IN INDIA

An interesting and provocative study of the biochemical changes in starvation is presented by J. P. Bose, U. N. De, and P. Mukerjee (*Indian J. Med. Res.* **34,** *143 (1946)*). The changes in blood sugar and oral and intravenous sugar tolerance were the most striking, although the authors also studied the concentrations of blood nonprotein and urea nitrogen, creatinine, uric acid, cholesterol, lipoid phosphorus, plasma chloride, serum calcium, and serum albumin and globulin.

The study was undertaken in Calcutta where many hungry Bengalese migrated with the outbreak of the Bengal famine of 1943. Two groups, totaling 84 patients were studied (those with renal disease and tuberculosis were eliminated). In group A, edema was absent or slight, although emaciation was present to an extreme degree. In this group starvation was of short duration, usually three to four weeks, and few had malaria or diarrhea. In group B, most had massive edema and many had marked ascites. These people had been chronic sufferers from malnutrition before the famine so that when the food shortage became severe they did not find street garbage inedible and were able to supplement their diet in this way. Most of them had malaria, pneumonia, dysentery, or anemia.

The fasting blood sugar (the Hagedorn-Jensen method was used throughout) was frequently low (45 to 80 mg. per 100 ml.) in both groups. In group A, 12 subjects had normal tolerance to the oral administration of 50 to 100 g. of glucose, while 15 subjects had a slow rise from 200 to 275 mg.

per 100 ml. in about two hours, where it remained through the three hour period. The other 22 cases were intermediate between the normal and this extreme. In direct contrast to the glucose tolerance in group A was that in the chronically malnourished, edematous individuals in group B; the fasting blood sugar was low, below 70 mg. per 100 ml. in all. Following similar quantities of glucose by mouth the blood sugar did not rise in any, remaining unchanged in 12, and actually falling by the end of three hours to about 40 mg. per 100 ml. in the other 16. When glucose (0.2 g. per kilogram) was administered intravenously to the individuals in group B, again two types of curves were observed. Beginning from a low fasting normal in 15 individuals, a peak of about 325 mg. per 100 ml. occurred in fifteen minutes, followed by a gradual fall to 200 g. per 100 ml. one hour after the injection, and in 12 individuals the peak was far less (175 mg. per 100 ml.) and returned rapidly (sixty minutes) to the fasting level, an essentially normal curve. Unfortunately, the intravenous studies were not carried on longer than one hour, and further, each of the two types of curves following oral and intravenous administration were not comparable as to individuals. The presence of "starvation diabetes" was clearly evident in both groups, although had only oral tolerance tests been used, diminished tolerance would not have been observed in group B, presumably because of poor absorption.

The total plasma protein concentration was slightly or in a few instances markedly reduced in all subjects, more so in group B. This was especially true of the albumin concentration which was between 2.5 and 3.5 g. per 100 ml. in group A, but between 1.0 and just over 2.0 g. per 100 ml. in group B. Thus, the albumin concentration was consistently lower in the group exhibiting edema. The globulin concentrations were normal or high in a few, mostly individuals with chronic disease such as kala-azar, malaria, and dysentery. The blood calcium concentration in all but 4 cases was reduced to between 7.5 and 8.5 mg. per 100 ml.

There was a moderate increase in blood nonprotein nitrogen in the majority of the subjects, considering the upper limit of normal as 35 mg. per 100 ml., although the blood nonprotein nitrogen did not rise above 50 mg. per 100 ml. The blood urea nitrogen was also elevated in many instances, but to a less extent than the total nonprotein nitrogen. In all instances the blood uric acid concentration was between 3 and 5 mg. per 100 ml., and was thus above the authors' normal of 3 mg. per 100 ml. The blood creatinine concentration, on the other hand, was normal in all cases.

The blood cholesterol was below 100 mg. per 100 ml. in all instances, while the majority of determinations of lipoid phosphorus (method of Bloor) were within the normal range, although a few were slightly elevated.

The majority of the results are presented by the authors without critical discussion; however, they are of sufficient interest to stand by themselves. Although the incidence of starvation diabetes was high as might be expected, one would like to know the reason for the failure to find hyperglycemia following the oral administration of glucose. If due to failure to absorb glucose, it was certainly complete or very nearly so.

The finding of widespread hypoalbuminemia is not a surprise, but its apparent direct relation to the incidence of edema is contrary to some of the studies made during World War II (*Nutrition Reviews* **3,** *225 (1945);* **4,** *28, 317 (1946);* **5,** *31 (1947)*). It would be difficult to understand the conflicting data from various laboratories and from different parts of the world in regard to the etiology of "hunger edema" unless it is postulated that several factors are responsible for its genesis. In the study of Bose, De, and Mukerjee the correlation of hypoalbuminemia and edema adds weight to the argument that the plasma albumin concentration is an important factor.

NOTES

Nutrition in War Prisoners

J. N. Crawford and J. A. G. Reid (*Canad. J. Research (Section E)* **25,** *53 (1947)*) reported nutritional disturbances in Canadian soldiers over a period of forty-four months, during which they were prisoners of war in the hands of the Japanese. The chronologic sequence of development of signs and symptoms is shown and the effect of dietary deficiency on the various systems is reported, with remarks on various aspects. The response to minimal treatment also is described.

Colorimetric Determination of Vitamin A

A. E. Sobel and H. Werbin (*J. Biol. Chem.* **159,** *681 (1945); Nutrition Reviews* **4,** *107 (1946)*) introduced glycerol dichlorohydrin (GDH) as a new color reagent for the determination of vitamin A. This has appeared to be a promising reagent, but little data on its use with biologic materials have appeared until recently.

A. E. Sobel and S. D. Snow (*J. Biol. Chem.* **171,** *617 (1947)*) reported on the application of the reagent to the measurement of vitamin A in serum. They found that vitamin A and carotene could be measured just as well in the presence as in the absence of the hemolyzed blood cells. The activity and stability of a number of preparations of "activated" GDH were tested and found to be reasonably uniform and stable. A comparison was also made of the relative effect of the presence of water on the Carr-Price and the GDH reagents. Whereas the Carr-Price reagent gave variations in reading with just a trace of water present, GDH gave exactly the same reading whether the vitamin A solutions were anhydrous or saturated with water, and in this respect it is vastly superior to the Carr-Price reagent. Carotene causes an interference with GDH just as with Carr-Price reagent but it is about 20 per cent greater in value. This is not a serious disadvantage since the amount of carotene generally found in serum can be corrected for as in the Carr-Price method as modified by W. J. Dann and K. A. Evelyn (*Biochem. J.* **32,** *1008 (1938)*). Sobel and Snow (*loc. cit.*) also found that by modification of the color estimation their method is as sensitive as the Carr-Price test.

The GDH method has been compared with the Carr-Price method on whole and saponified serums; values with GDH on either whole or saponified serums compare favorably with Carr-Price figures on saponified serums, so another advantage of the GDH reagent is that the saponification of the serum sample is unnecessary.

The method as developed with the GDH reagent thus appears to be the most useful colorimetric method devised for vitamin A, bearing out the promise suggested by the first papers of Sobel and Werbin (*loc. cit.*).

Strepogenin in the Protein Molecule

Earlier knowledge concerning the peptide-like growth factor named strepogenin has already received attention in this journal (*Nutrition Reviews* **4,** *273 (1946);* **5,** *218 (1947)*). Sufficient new information has been obtained to warrant a further communication by D. W. Woolley (*J. Biol. Chem.* **171,** *443 (1947)*). A free amine group has been recognized as a characteristic of strepogenin (D. W. Woolley and H. Sprince, *Federation Proceedings* **4,** *164 (1945)*). If this amino group is free in the protein, then treatment of the intact protein with reagents which act on amino groups should result in products which do not give rise to strepogenin when digested with trypsin or with acid, though if the strepogenin should not be at the end of the peptide chain of the molecule, such treatment should not change the strepogenin potency.

F. Sanger (*Biochem. J.* **39,** *507 (1946)*) demonstrated that insulin has a free amino group and has assayed insulin for strepogenin activity. Before assay for strepogenin the

dinitrophenyl insulin was digested with trypsin in a series of experiments and the effect compared with that of hydrolysis by cold strong hydrochloric acid. Such acid hydrolysis of casein and insulin yields strepogenin activities one-fourth as great as those obtained after trypsin digestion.

Trypsinogen and casein, two other proteins rich in strepogenin, have been examined by Woolley: in trypsinogen, as in insulin, potency was destroyed by alteration of the amino groups, but in casein about half was retained after such procedures.

The tentative conclusions that appear to be justified by these observations are that some of the free amino groups of the protein belong to strepogenin, and that glycine is its amino acid end group.

In a later communication Woolley (*J. Biol. Chem.* **172,** *71* (*1948*)) gives some evidence regarding the strepogenin activities of various pure compounds prepared by him. True strepogenin potency was observed only in serylglycylglutamic acid, glycylalanylglutamic acid, and glycylglutamic acid. Several similar compounds in which the amino group was substituted were inactive.

This evidence is still to be regarded as suggestive rather than indicative of the precise chemical structure of strepogenin, since the most active pure compound prepared was only one-fortieth as active as the best concentrate of natural strepogenin from casein or insulin.

Scorbutic Symptoms in Vitamin A Deficient Rats

Known instances in which one vitamin influences the behavior or action of another vitamin were reviewed a few years ago by T. Moore (*Vitamins and Hormones* **3,** *1* (*1945*)). A clearcut instance of this is contained in a note by J. Mayer and W. A. Krehl (*Arch. Biochem.* **16,** *313* (*1948*)). These workers produced vitamin A deficiency in rats by feeding a vitamin A free diet containing sucrose, casein, corn oil, mineral salts, and crystalline vitamins. Many of the symptoms observed—bleeding of the lachrymal glands, swollen gums, swelling of joints, and early paralysis of the forelegs—resembled those of acute scurvy. A marked and rapid improvement in the condition of the animals, particularly in those symptoms involving bleeding and paralysis, was obtained after intraperitoneal injection of massive doses (50 mg.) of ascorbic acid.

Previous workers (for example, G. Jonsson, A. L. Obel, and K. Sjöberg, *Z. Vitaminforsch.* **12,** *300* (*1942*)) had reported that vitamin A deficient rats exhibit lowered blood and tissue levels of ascorbic acid. The present workers confirmed these results, and found also that the adrenal glands of their deficient animals were hypertrophied, with evidence of adrenal hemorrhage. Changes in the adrenals are characteristic of ascorbic acid deficiency.

The authors emphasize that ascorbic acid is not alleviating "true" symptoms of vitamin A deficiency in these animals, but alleviates only those symptoms associated with the simultaneously produced ascorbic acid deficiency. Administration of this latter vitamin may be necessary to permit observation of the symptoms of "uncomplicated" vitamin A deficiency.

Clinical practice has emphasized that nutritional deficiencies of a single vitamin are rarely found in man, since the poor diets characteristic of vitamin deficient patients are usually lacking in several food factors. In the present instance, evidence indicates that a deficiency of one vitamin may actually precipitate a deficiency state due to lack of an entirely different food factor, which, according to ordinary standards, is present in adequate amounts, or, as in this case, not ordinarily required in the diet.

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